

14 February 1980

A dearth of new drugs

THE Californian court case in which Mrs Betty Mekdeci is trying to establish that *Bendectin*, an anti-nausea drug manufactured by Richardson Merrell, was responsible for the deformities with which her son was born, can hardly have come at a worse time for those who thought they could at last see some light at the end of the tunnel of regulations in which the pharmaceutical industry has found itself since the thalidomide tragedy of 1961. Whatever the outcome of the case against *Bendectin* the blaze of publicity surrounding it is bound to make this a less than opportune moment for the political turn-about on regulations that seemed to be imminent, at least in the UK. Therefore it is important that the *Bendectin* case is used to illuminate rather than to obscure the arguments for and against relaxing regulations.

The pharmaceutical industry is prone to claim that were the regulations of the American Food and Drugs Administration to apply worldwide, no new drug would ever reach the market. Although that is hyperbole, it is true that regulations are more restrictive in the US than even in the UK which has the stiffest regulations in the European Economic Community. It is also true that the figures underlying the claims are dramatic. Compared to 20 years ago, it now takes about three times as long from the discovery to the marketing of a new drug. The demands for drug testing before marketing have risen enormously, some would say 100-fold. The number of new drugs that reach the market each year has been halved. The cost of developing a successful drug now varies between £3-5 million. For every drug that reaches the public, several will have failed at the stage of clinical trials and many hundreds will have fallen by the wayside at an earlier stage.

Not too many tears need be shed in response to these facts. Drug testing by the industry used to be inadequate in both quantity and quality. In the immediate aftermath of thalidomide, better testing was either adopted by or forced upon the industry. But instances of inadequate testing continued to come to light thereafter and even if those instances were the whole, rather than the tip, of an iceberg, they provided some justification for the increasing stringency with which the whole industry became regulated. There has also been justification for the mandatory introduction of new and more sensitive methods of testing whatever the costs to industry.

Without a doubt the new drugs which now reach the public are safer than they used to be. The fact that there are fewer of them not only reflects the earlier detection and removal of hazardous drugs but also the growing and justifiable bias by regulators against "me-too" drugs, which ape the successful product of a rival, and in favour of truly innovative products.

On the other hand, regulations have had an irresistible tendency to breed further regulations because, faced with the possibility that lives are at stake, any regulatory body will understandably play safe. If a better, or even just a supplementary, test can be added to those already in force, it is safest to insist that it be carried out. Would it not be better to ask for the drug to be tested in an additional species? Why not replace a three month drug dosing period by one of a life time; and then

why not insist on testing for any effects on the generation—or two?

By playing safe, the regulators are protecting themselves in the event of any unforeseen tragedy as well as protecting the public. It is, however, possible to be over-protective towards the public. Regulations designed to keep out the bad can also keep out, or at least hold up, the introduction of the good. That is what the pharmaceutical industry claims has happened to an unreasonable extent.

Much of their venom is directed against the bureaucrats. Why, they ask, does it take months for the UK Committee on Safety of Medicines to decide whether or not to grant permission for a clinical trial and up to a year for approval of a marketing licence after the clinical trial has been completed? (To which a partial answer is that it takes a long time to sift through the amount of evidence that is currently demanded and even longer when the evidence is inadequate or badly presented). More fundamentally, the industry and much of the medical profession, argue that, as a way of testing for hazards, animal testing and even clinical trials have limitations which are not adequately recognised. As a result too much time and effort, not to mention money, is devoted to trying to avoid what is unavoidable at that stage.

Animal tests will never provide a complete guide to human reactions to a drug and a clinical trial can never be on scale that is sufficient to detect side reactions of low incidence. It is argued that the path of a new drug both up to and through the clinical trial stage should be made easier. In return more attention should be paid than at present to the post-marketing stage. In other words the public should opt for taking more risks because of the greater benefits that such a decision would bring, on balance.

The general line of argument in favour of relaxing regulations is persuasive, but should only be met with a cautious practical response. There may be a case for cutting back on animal testing but if so, it is only on quantity in a limited number of respects and not at all on quality. There probably is a case for allowing and encouraging regulatory bodies to give a rapid, provisional go-ahead for companies to mount a clinical trial or even to market a new drug on the basis of a summary of the evidence before it is examined in full. But that procedure should be reserved for really innovative drugs and should not remove either the obligation for the company to provide the full evidence or for the regulators to examine it and, if necessary, to withdraw their provisional permission.

Finally, better ways will have to be devised for the monitoring of drugs once they are in full scale use. Although this is widely admitted in principle, there is little doubt that it will be resisted in practice, not least because it is likely to involve a cumbersome bureaucratic mechanism. However, had an adequate monitoring system already been in operation, we would surely have been spared, in one way or another, the need for the dramatic and emotional court room drama that is currently bringing anguish to the many women who took *Bendectin* early in their present pregnancy. □



United States

Universities push for creation of advanced energy research centres

US universities are pressing the Carter administration to help set up a number of university-based advanced energy research centres, concentrating on areas of interdisciplinary research that currently fall between the mission-oriented R&D programmes of the Department of Energy and its basic research commitments.

In his budget request to Congress for the fiscal year 1981, the President has suggested that \$3 million be allocated to the department to support the initiation of such centres, an idea formally suggested only last October by Dr David Saxon, president of the University of California.

The universities hope that the administration can be persuaded to contribute more than \$150 million over the next three years both to establishing 15 such centres, and to provide a national support programme of grants, traineeships and fellowships.

Administration officials are approaching the scheme cautiously, however, pointing out that the value of such centres should be judged on their scientific merits — and sceptical of any demands to institutionalise university research efforts.

The decision to provide the initial funding for a number of centres, albeit at a much lower level than the \$15 million Dr Saxon had suggested, reflects a growing desire within the Department of Energy to emphasise the potential role of universities in carrying out what is referred to as "goal-oriented basic research".

In the 1981 budget request for the DoE Office of Energy Research, for example, funding for university-based energy research is scheduled to increase by 22%, a real growth of about 12%; in particular, "university research support", which includes the new interdisciplinary centres, would more than double, from \$7.1 million to \$14.3 million.

The specific proposals made by Dr Saxon were backed up by a report which he commissioned last August from the Association of American Universities. Prepared by a committee under Dr Jack Hollander, associate director of the University of California's Lawrence Berkeley Laboratory, the report was on Dr Saxon's desk within five weeks, and passed to Washington shortly after.

The main foci of the report are claimed gaps in the department's research strategy — and the way that universities might help to fill them. In particular, the report claims that the heavy emphasis within major R&D programmes on short-term technology development "inhibits the pursuit of goal-oriented basic research and the interaction

of university researchers with the department".

In general it says that "much goal-oriented basic research tends to be considered too basic by the DoE mission-oriented programmes, and too applied by the DoE/OER basic science programmes". Examples of such research gaps, it suggests, can be found in fields such as synthetic fuels and nuclear waste disposal.

In transmitting the report to Dr Frank Press, director of the Office of Energy Research, and Dr John Deutch, undersecretary for energy, Dr Saxon spoke of the "urgent need for very substantial improvement and expansion" in the department's research strategy.

Building on the AAU report's conclusions, Dr Saxon suggested that the department agree to initiate support for five centres a year over the next three years, each centre receiving \$2 million for "core support" and \$1 million for scientific equipment.

Centres would be selected on the basis of a national competition. Each would build a "coordinated, coherent research programme around a central theme or subject"; styles would vary from new interdisciplinary laboratories in technical fields (such as the Materials Research Laboratories established by the Department of Defense) to interdisciplinary graduate study and research groups, such as the Energy Research Laboratory at the Massachusetts Institute of Technology.

Others might develop strong

programmes applicable to a variety of energy technologies — such as research on high temperature chemistry applicable to nuclear, solar, fossil and geothermal technology — or involve research on the biomedical and social sciences related to energy supply and demand.

The proposal was speedily processed by the DoE, and submitted in late December to the Office of Management and Budget as a last minute addition to the 1981 budget request. OMB agreed to provide \$3 million for the scheme — expected to be enough to get four or five centres started — but insisted that further money would only be available if proposals are of a sufficiently high standard.

Dr Edward Frieman, director of the Office of Energy Research, told the Energy Research Advisory Board last week that proposals would have to demonstrate some special qualities, such as a strong interdisciplinary basis or close links with private industry, to qualify for funding.

"We do not want to see just a repackaging of our support for basic energy sciences", Dr Frieman said. "If the quality of the proposals is not high enough, I will not fund the programme".

Several major hurdles face the energy centres scheme, in addition to the scepticism of budget officials. One is the inevitable political infighting over where the first centres should be situated, given the traditional desire of Congressmen for 'equitable distribution' of federal dollars between geographical regions.

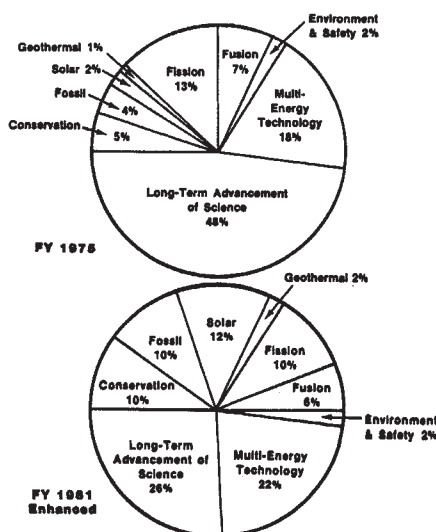
A second is the response of the national multipurpose laboratories supported by the DoE to a proposal that would seem to shift yet more research funding away from the laboratories into the university system. The department's budget proposal for 1981 is that the universities' share of OER funding should increase from 22% to 24%, with a corresponding reduction in the proportion of support from the national laboratories (although the latter have been provided with \$60.3 million to improve basic facilities and their net funding will still increase).

Energy department officials, however, have currently taken as a key policy target the need to establish an adequate "technology base" for all its various energy-supply activities; and argue that, taking a leaf from the Defense Department's book and echoing the AAU, this can partly be achieved by emphasising goal-oriented basic enrolments research.

Universities, facing declining student enrolments and contracting research possibilities elsewhere, are keen to fit the bill. In the words of Dr Saxon: "A new programmatic departure . . . will signal the deliberate mobilisation of scientific talent in universities for the assault on energy problems of all sorts."

David Dickson

Shift in emphasis in Office of Energy Research support for basic energy sciences from 1975-81



Fast breeder reactors delayed until after 2000

A declining enthusiasm for nuclear power in the US has led the Carter administration to stretch out still further research and development efforts leading to the deployment of fast breeder nuclear reactors. These are not now planned to come into commercial use until well beyond 2000.

In his budget request to Congress two weeks ago for the fiscal year 1981, President Carter reaffirmed his previous determination to halt construction of the liquid metal fast breeder demonstration project at Clinch River in Tennessee, a move so far resisted by Congress.

At the same time, however, the President has also recommended other broad cuts in the breeder development programme not proposed in previous years. This is in line with a decision taken late last year to stretch out the LMFBFR programme and defer building breeder reactors, previously expected to come into use in the 1990s.

Administration officials are thought to believe that, given the current uncertainties surrounding the future of nuclear power, following the Three Mile Island accident, as well as adequate domestic supplies of uranium, it would probably be impossible at present for a new reactor type to be produced and licensed in the US. The Department of Energy had asked the White House to keep breeder funding at the 1980 level, apart from Clinch River.

Recommended cuts in the breeder programme include reducing support for LMFBFR technology from \$140 million to \$80 million in 1981, and cutting out completely further development of the gas-cooled breeder reactor, currently being supported as a back-up programme.

Funding for research and development in fuel processing technologies for breeders is proposed to be reduced from \$49.5 to \$12 million, and plans for a conceptual design study for a larger scale plant than the Clinch River project will be terminated.

Overall funding for the breeder programme will, if Congress agrees, be cut from \$761 million to \$384 million. What actually happens, however, will depend on the outcome of the conflict over the Clinch River funding; until this is resolved, the administration is facing severe difficulties in producing a balanced programme for nuclear fission research.

Domestic cutbacks in the breeder programme have heightened the enthusiasm of energy officials for cooperation with other countries. In particular an exchange of agreement was signed with France at the beginning of December, through which it is hoped to work with French scientists and engineers. □

Canada

Election candidates asked for science funding commitments

Canadian scientists are mounting an active campaign to inform candidates in next week's general election of the need to increase support for university research. **David Dickson** reports

SOON after Canada's nine-month-old Conservative government resigned last month, scientists at the University of Toronto placed a letter on campus noticeboards asking for volunteers to investigate the attitudes of local candidates in next week's general election towards research funding.

Each volunteer is being equipped with a background kit on federal R&D policies prepared by groups such as the Canadian Association of University Teachers and the Canadian Federation of Biological Societies, and then assigned to candidates in one of Toronto's 23 ridings (constituencies).

The idea is to discover whether in general terms a candidate is prepared to support increased funding for research and development — or to provide information to those who have not considered the issue before — and also to report back on any commitments that a candidate may make.

These are checked back with the candidate's party, and noted for future use. "We are using this as a device to wheedle commitments, and if we get the commitments we are going to nail them," says Dr Ron Clarke, director of the University of Toronto's Office of Research Administration.

The scientists' campaign is being organised jointly by the CAUT, the CFBS, and other professional societies such as the Canadian Association of Physicists. With lobbying planned in more than 50 election districts, it is the latest tactic in an aggressive grass-roots strategy waged over the past few years to focus political attention on the relative lack of funding for university research.

Argument has been focused on the fact that government spending on basic research fell from 0.1% to 0.07% of the gross national product between 1969 and 1979, less than half that of most industrialised nations.

Responding to such criticisms, the Liberal government made a commitment to increase overall R&D spending to 1.5% GNP by 1983 shortly before losing power last year; and in November, Mr Howard Grafftey, Minister of State for Science and Technology, announced that the Conservative government proposed to increase the funding of the Natural Sciences and Engineering Research Council by 32%, to a total of \$159.8 million, in the fiscal year 1980-81.

This increase was contained in the total budget figures over which the Conservative government was defeated in Parliament in December. But last week, with the election campaign in full swing, Mr Grafftey announced that NSERC's budget would be increased still further, to a total of \$162.6 million.

At the same time, Mr Grafftey announced that the budget of the Social Sciences and Humanities Research Council would be increased by 16.2%, and that of the Medical Research Council by 17.4% bringing the total increase for the three research councils to 26.4%.

Budgetary levels for the councils approved by the Cabinet are for the first year of five-year plans which each has submitted. The increased funding has been welcomed by the scientific community, which feels that, whichever party wins next week's election, it will be difficult to go back to the lower increases previously projected. But there is concern over whether the increased level of funding will be maintained into future years.

The Party has publicly committed itself to an eventual target of spending 2.5% of the gross national product on R&D, although without being specific about timing. The small but growing New Democratic Party — which could hold the balance of power in a close ballot — has adopted the ambitious target of 4% by 1990 and 2.5% by 1983.

Many scientists doubt whether these are realistic targets, given the rapid sustained growth in funding that they would require. But there is determination to keep pressure on the new government to confer stability to the Canadian research effort.

One element of concern is that the new government should retain a minister with full-time responsibilities for science and technology. Another is that the provinces should play their part in the national effort to stimulate R&D; some have recently been criticised for diverting federal funds allocated — although not mandated — for the support of further and higher education.

In Toronto, the scientists claim their grass-roots lobbying towards such objectives is beginning to pay-off. Already one Liberal ex-Cabinet member has said his party, if elected, will appoint a full-time minister for science and technology. But what actually happens after election day remains to be seen. □

Poland

Science planning praised — but improvements still needed

SCIENCE planners and civil servants in the science-related ministries should improve their scientific qualifications, according to the "guidelines" for the Eighth Congress of the Polish United Workers' Party this week.

The "guidelines", which after due discussion will form the basis for the 1981-85 Five Year Plan, initially paint a glowing picture of successes scored by Poland's special approach to science planning, with its hierarchical structure of research tasks, from "Government" and "Key" down to "Departmental" problems. These achievements range from laser thermonuclear microsynthesis to mining tectonics, the mapping of Poland's flora and fauna, and the use of "Arctic Marine organisms" (ie krill) as a source of protein.

But it does seem that the "problem solving" structure of Polish research has not yet overcome the difficulty endemic throughout the Comecon bloc — that of implementing R & D results.

"It is absolutely necessary", say the guidelines "to introduce a system enabling universal incorporation of Polish inventions in national research and development endeavours. Contacts and cooperation among scientists, engineers and workers must be strengthened as an important vehicle of propagating technical progress and enriching the scope of innovations in industrial plants."

When it comes to the details of research planning, however, the "guidelines" show a certain ambivalence. On the one hand "the realisation of long-range research plans requires their closer connection to

development programmes of the corresponding fields of the national economy". On the other, "more flexible methods of work must be developed in scientific as well as research and development units", and "the manner in which scientific research establishments are organised, their employment structure and planning methods, must be adjusted to changing needs."

To enable them to strike the proper balance between these demands, the staff of the relevant ministries are urged to continue their higher education "in cooperation with the institutes of the Polish Academy of Sciences and university-related schools".

The "guidelines" propose a number of "tasks" for the special attention of the science and technology planners. These come as no surprise, either within a world context (limitation of petroleum consumption, development of "unconventional" energy sources, and environmental protection) or within the special conditions of Poland (water management, development of high-protein and oleaginous plants, the development of specialised exports to the highly developed countries, and the export of Polish technical know-how).

But perhaps the most interesting "task" is "the production of pharmaceuticals . . . through the more extensive use of domestic syntheses and achievements in the field of genetics", a field in which, according to a recent article in *Polityka*, the commercial breakthrough point may be expected in the near future.

Vera Rich

United Kingdom

Study finds malnutrition in 7% of elderly

NUTRITIONAL problems among Britain's elderly population are highlighted in a report* published recently. "Nutrition and Health in Old Age", is the result of a survey of the nutritional status of 365 old people in 1972-73. The incidence of malnutrition was 26 (7%). It was twice as common in subjects over 80 years old.

Malnutrition was associated with both medical and social risk factors. Medical conditions included chronic bronchitis, dementia, depression, difficulty in swallowing, and poor dentition. The social risk factors included being housebound, living alone without regular cooked meals,

bereavement, and being dependent on welfare benefits — though the report does say that "lack of money did not appear to be the sole cause of undernutrition."

Among the 26 were three with scurvy, two with osteomalacia, and 13 with nutritional anaemia associated with haematological evidence of iron, folate, or vitamin B₁₂ deficiency. The remaining cases showed evidence of multiple nutrient deficiency. Diagnosis was drawn from clinical, dietary, biochemical and haematological studies; single clinical signs and isolated biochemical findings were considered to be unreliable.

The subjects were not a random population and the conclusions cannot be extrapolated to the population as a whole.

Alastair Hay

*Nutrition and Health in Old Age. Report on Health and Social Subjects, Volume 16. DHSS. Available from HMSO at £5.25.

The Netherlands

Recombinant DNA research thrives

RECOMBINANT DNA research in The Netherlands is expanding at a rapid rate. At the end of 1976 only seven projects at universities had been registered by the commission of experts, established by the Royal Academy of Sciences in 1975 to look at genetic manipulation. By the end of 1979, there were 78 such projects, of which about 10 are with industrial companies, such as Unilever Research. All this research, according to the commission's latest annual report just published, will take place in category I and II laboratories.

The work of the Academy's commission has now been taken over by a government commission whose members include representatives of trade unions, the health authorities and industry. The Academy commission relaxed the safety guidelines in 1978. These came into effect a year ago and were extended to include yeast as a host organism. The new commission has already announced that it will consider a further relaxation of the regulations.

The chairman of the first commission — in a farewell speech — emphasised the need to build a category III facility which researchers say is needed in the country. The lack of such a laboratory has resulted in a falling behind in important research on, for example, cancer-inducing viruses and genetic aberrations, fields in which until recently The Netherlands had made progress. Even with a further relaxation of the regulations, Dutch scientists say that a category III laboratory is essential for research using DNA of warm-blooded animals and, in particular, man.

The previously strict rules in the country did not result in the departure of Dutch scientists to work abroad. Only seven researchers left temporarily to work in a category II or III facility, and five of them also asked foreign laboratories to work for them. But, in its last report, the commission says that it would be wrong to conclude that molecular biological research in The Netherlands had not suffered from the strict guidelines in force until recently.

In The Netherlands, especially in industrial circles, there is still a lot of dissatisfaction about the conditions in which molecular biologists must work. The public debate which reached a peak in June 1978 has now died down, and among scientists there is very little opposition to the relaxing of the guidelines. A long-awaited special committee to study the ethics and social aspects of work with genetic material has still not been set up by the Dutch government, but this is expected soon.

Casper Schuurung

Dioxin

Chemical company study shows no dioxin hazard

A REPORT to be published this month claims that workers exposed to significant quantities of the animal carcinogen 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) over thirty years ago have a normal incidence of cancer, and a reduced incidence of cardiovascular disease.

The report, published in the American *Journal of Occupational Medicine*, is an epidemiological survey of workers exposed to dioxin at the Monsanto Chemical Company's site at Nypro, West Virginia. 228 men are known to have been exposed to the chemical: 117 as a result of an accident in the 2,4,5-trichlorophenol plant on 8 March 1949, and 111 in the routine manufacture of the herbicide 2,4,5-T. It documents the mortality indices for 121 of the 228 workers who were traced.

Acknowledging that these results are only part of the story, the report's authors — Judith Zack, an epidemiologist employed by Monsanto and Raymond Suskind, Director of the Institute of Environmental Health at the University of Cincinnati Medical Centre — say that details about the other workers will be published in due course.

Publication of this information by Monsanto is a welcome departure from the company's previous position of treating all information about these workers as

confidential. Little was known about the Monsanto accident until July 1976 when a trichlorophenol reactor at the Italian town of Séveso overheated, discharging dioxin over the town and its environs. In the aftermath of Séveso, details began to emerge of at least 16 similar accidents in various parts of the world. Accounts of these accidents and their consequences were requested by Italian officials and scientists anxious to ascertain the consequences of the dioxin exposure for the people of Séveso. Sadly, few companies have been forthcoming; this latest initiative on the part of Monsanto may have the effect of breaking the log jam.

A Monsanto spokesman described this latest report as "reassuring". But it is unlikely to allay all fears about the toxic properties of dioxin. For the results of the Monsanto survey differ from those following surveys of workers exposed to dioxin in West Germany and Holland. Workers known to have been exposed at Badische Anilin und Soda Fabriks' Ludwigshafen site in 1953 are reported to have suffered from a high incidence of gastrointestinal cancer, whilst others contaminated by the chemical at the Amsterdam site of Philips Duphar in 1963 are recorded as having a higher incidence of heart attacks (*Nature* 19 January 1978, p202).

Zack and Suskind note that their results do not agree with those obtained for German and Dutch workers. They also acknowledge that their survey does not confirm the higher than normal incidence of bronchiogenic carcinoma in workers exposed to dioxin in an accident in Czechoslovakia in 1964.

More recent evidence about the potential carcinogenic properties of dioxin are reported in a survey of Swedish forestry workers published in the *British Journal of Cancer* 39, 711; 1979. According to its authors, Dr Lennart Hardell and Anita Sandström of the Center of Oncology at the University of Umea, exposure to dioxin contaminated 2,4,5-T or chlorinated phenols could be responsible for the six-fold higher incidence of soft tissue sarcomas seen in these men.

With so much conflicting evidence about the long-term effects of exposure to dioxin, the results of the Monsanto survey — albeit the largest so far — are unlikely to still fears about the risk posed by the chemical. The US Environmental Protection Agency has instituted a temporary ban on 2,4,5-T. Whether or not that ban should be made permanent will be decided after an Agency hearing on the herbicide due to begin on 11 March. The EPA will have to make sense of this conflicting data. **Alastair Hay**

Mediterranean

No cash to clean up pollution

MEDITERRANEAN states have come up with only one third of the money needed to maintain the United Nations Environment Programme's Mediterranean pollution programme, UNEP's deputy director Peter Thacher announced in Barcelona on Monday.

Speaking at a meeting of the 17 signatories of the 1975 'Barcelona convention' on pollution in the Mediterranean, Mr Thacher warned "we have today only seven weeks left before we must stop" a number of the programme's activities. Jobs in the programme's 12-strong secretariat are also threatened: "a number of critical posts are filled on contracts which will expire in March unless new cash contributions are received", said Thacher.

A year ago, a meeting of the signatories in Geneva agreed to establish a trust fund of \$6,480,000 to guarantee the programme for the two years 1979-80. The 17 states, plus the European Commission, were to contribute half, UNEP one quarter, and other UN agencies one quarter in goods and services. A year later, eight countries and the EEC had contributed nothing.

Only five countries have contributed in full, and these were among the smallest and

poorest: Algeria, Tunisia, Cyprus, Malta, and Greece, providing \$130,000 among them.

Among the major countries France has been the most forthcoming, with \$990,000 of its agreed \$1,564,000. Italy and Spain, however, which committed themselves at Geneva to spend \$744,000 and \$412,000, have so far contributed nothing, said Thacher. In total the countries have contributed \$1,150,000 against an expected \$3,200,000.

The next treaty to be signed is the treaty on land-based sources of pollution, the crown jewel of the Mediterranean programme. It was expected that states would sign the protocol in Athens this May and ratify it within two years, but, said Thacher, there were not even enough funds in the kitty to convene the conference.

Moreover land-based sources, particularly of untreated sewage, are the major cause of pollution in the Mediterranean, and would cost — according to rough estimates — some \$15 billion to clean up. UNEP officials have been assuming that this money would be spent over some 10-20 years, representing a \$1.5 billion to \$750 million-a-year spend, 500-1000 times the present — and

unachieved — target.

The 'Blue Plan', a proposed high-level economic and sociological study of the likely development of the Mediterranean region to the year 2000, is also threatened, though not by lack of cash. States had been asked last February to nominate two candidates each for six posts to implement the Blue Plan, but two months past the deadline only seven nominations have been received from seven countries. And, said Thacher, "very few of these candidates have sufficient academic qualifications or experience". He concluded that governments were "not serious" about the plan.

● In an extension of the UNEP Regional Seas Programme (of which the Mediterranean programme is a part) to the Caribbean, experts from 23 mainland and island states of the region have agreed to recommend to their governments an action plan on pollution and development. "In its economic and social aspects, the Caribbean Action Plan goes well beyond the Mediterranean Action Plan" says UNEP. The first governmental meeting on the plan will be held in September.

Robert Walgate

NEWS IN BRIEF

US city bans nuclear waste shipments

THE city of Missoula, Montana (population 58,000) banned last week all shipments of nuclear materials through the city limits. Citing two accidents involving trucks carrying nuclear wastes that occurred this winter on Interstate 90 — which runs through the city's northern boundary — as evidence of the hazard involved, the City Council passed the ban by a vote of 7:1. The measure is expected to interfere with the shipment of wastes to the Hanford nuclear plant in eastern Washington. Included in recent shipments to Hanford are wastes from the accident at Three Mile Island in Pennsylvania.

Séveso director killed by political group

THREE men and a woman, ambushed Paolo Paoletti, aged 39, a director of the ICMESA chemical plant in Séveso outside his Monza home last week. The Italian urban guerrilla group, Front Line, claimed responsibility for the assassination. Paoletti was one of several senior ICMESA officials arrested and charged with operating a dangerous factory after four pounds of dioxin were released when a chemical reactor exploded in the factory in 1976. More than 600 children developed acute cases of chloracne as a result of exposure to dioxin. The legal case is still pending with the parent company Hoffman-La Roche of Switzerland expected to admit responsibility for damages caused, but not negligence.

UK radiation study overestimates safety

A BIENNIAL radiation survey conducted by the UK Health and Safety Executive has seriously underestimated the exposure to the workforce at 11 UK nuclear power stations by its exclusion of part-time workers from the study. Although claiming that none of the 7500 nuclear plant workers in either 1977 or 1978 received more than the international limit of 5 rems, the report did not cover an estimated 1500 temporary employees, many of whom were hired to do the hazardous work of pipe delagging in areas heavily exposed to radiation. It is not uncommon in pipe delagging operations for workers to receive their allowed quarterly dose of 2.5 rem in less than two weeks. These workers are then replaced by other temporary employees. An HSE official confirmed that the study did not cover "employees not directly employed by the Central Electricity Generating Board" and said that no figures were available on the numbers hired to do delagging or on the

radiation doses they had received. *Health and Safety: Nuclear Establishments 1977 — 1978 HMSO £1.25.*

More UK nuclear cracks

BOTH gas cooled reactors at Britain's Bradwell nuclear plant in Essex have been shut down to investigate cracks in the welds of the expansion bellows of the primary cooling circuit similar to the ones found in the Dungeness reactors two weeks ago (*Nature*, 17 January, page 238). The first Bradwell reactor had been shut down for routine maintenance when the cracks were discovered. As a safety measure the second reactor was shut down and similar cracks were discovered there as well. The reactors could be off line for a year or more.

UK centre for analysis of technical change

A NEW research centre for analysing technical change with the goal of "increasing national wealth" is to be set up by the Leverhulme Trust in conjunction with the Science Research Council and the Social Science Research Council. Leverhulme will contribute £1.5m towards the centre and the two research councils will each contribute £½ million over a period of the first five years followed by £250,000 a year for the second five years. Sir Michael Swann, FRS, will chair the organising committee and the centre's governing board. The types of issues to be considered include energy and material resources, manufacturing technology and unemployment. The majority of the proposed staff of 20 professionals and 10 supporting staff will be on secondment or will have joint appointments with universities, companies or government establishments.

Vietnam veteran records Agent Orange testimony

CHARLES HARTZ, a US Vietnam war veteran, who was exposed to the dioxin containing defoliant Agent Orange, initiated a massive law suit against the manufacturers of the chemical in Philadelphia last week. Hartz, who suffers from terminal brain cancer, videotaped his testimony accusing the manufacturers of negligence in not testing the chemical for its effect on humans. The pre-trial session had been organised by Hartz' attorney out of fears that Hartz will not live to testify when the trial begins later this year. In spite of objections from the manufacturers, District Judge George Pratt ruled that video tape was the best way of preserving Hartz' testimony. The law suit against Dow, Monsanto, Hercules, Diamond Shamrock and Thompson Heyward is presently being organised by 3000 exposed veterans but is still open to others.

20,000 demonstrate against Brittany reactor

TWENTY thousand demonstrators carrying French and Breton flags braved wind and rain squalls to march from the town hall in Plogoff to the site of a proposed nuclear reactor on Cap Sizan on the wild Brittany coast. Called to form part of a continuous protest to six weeks of hearings lasting until 14 March, the demonstrators plastered the town with slogans including a reminder to the government of its promise of April 1974 that "nuclear power will not be imposed on a population that refuses it".

Sussex physicist appeals to Queen over job

DR ROBERT Golub, a 42-year-old American physicist working at the University of Sussex, has appealed to Her Majesty the Queen over the termination of his research fellowship. The Queen is 'visitor' to the university, a role following from its charitable status; in principle she acts as an ombudsman, caring for "breaches of the rules of natural justice" (in the words of one legal expert) but not for matters of academic merit.

Golub's research fellowship, which is paid for by the Science Research Council, has been renewed regularly since 1968 for his work on ultra-cold neutrons. A new neutron source he devised, using superfluid helium, is to be installed at the Institut Laue Langevin, Grenoble, for 700,000FF (£80,000), but without his assistance. Golub believes that an industrial tribunal would dismiss his case because of a clause in his contract which waives his redundancy rights.

The Privy Council has written to Golub telling him that it could intervene only if the university was guilty of "incorrect procedures or improper motives".



"I don't think you've much chance! Look who's in front of us in the queue!"

FEATURES

Low expectations for Swedish PhDs

In Sweden, as in most European countries, young scientists are facing problems finding work. **Wendy Barnaby** reports on the falling market in research positions for newly-qualified PhDs

THE expansion in university teaching in the 1960s has not been followed by the creation of research posts for today's post-graduates, and many young PhDs in the natural sciences are finding it very hard to do research.

The expectations of young Swedish scientists have certainly fallen, yet they are hardly ever — strictly speaking — unemployed. According to Dr Bengterik Ronne, at the central administration of universities and tertiary education institutions (UHÄ), PhDs can still get jobs but they have to start at a lower level than they would have done a few years ago. This is borne out by figures published by the National Central Bureau of Statistics, showing unemployment amongst all licentiates (a qualification, now abolished, a little lower than a PhD) and PhDs, three years after graduation.

The latest available statistics cover the period 1976–1978, and continue a reasonably unchanging pattern in which most PhDs (as high as 90% to 98%, for this period) say they are employed wholly or partly within the field for which they were educated, with relatively qualified tasks. Only eight people over the whole period, out of a total of 1,008, said they were unemployed, and there is no way of knowing from the statistics whether they were licentiates or PhDs, or whether they had read social or natural sciences. But the statistics hide a lot of discontent.

Not many PhDs go into industry. "In the 1960s, Swedish industries were very optimistic about the numbers of PhDs they would need", says Professor Ingvar Lindqvist, Secretary of the Natural Sciences Research Council (NFR); "but when economic conditions changed for the worse, they realised they had overestimated their needs, and both industry and government lost interest in PhDs." According to Bengt Gustafsson at the central organisation of the academics' trade union (SACO/SR), there are only 700 PhDs working in Swedish industry, and most of them are chemists doing research in pharmaceutical companies.

Most natural sciences PhDs, then, end up in universities. But conditions there for them are far from good. The 185 who received their doctorates in 1976/77 had less than a one in ten chance of getting a research position (*forskarassistent*), which can be held for two three-year periods. A

small number of PhDs are given grants by the NFR and hope to be supported by NFR funds for their entire careers.

Other options are to apply for grants from other foundations or become attached to research projects that are already funded. But all grants are very insecure, as Professor Lindqvist complains. "There is a general political philosophy in this country of security in jobs", he points out, "but it hasn't worked well at the universities. People get grants for one year at a time."

Under the security of employment laws in Sweden, a university is obliged to offer a research scientist another job at the same salary — either in his own department or in another one — if his grant dries up. But trying to find niches for displaced people can be a difficult problem, as one of the SACO/SR representatives at the Royal Institute of Technology (KTH), Dr Bertil Wilner, has found. "It's easier to find alternative positions for technicians and administrative staff", he says, "but for specialised research scientists it's a very hard problem to solve." He estimates that about 5% of KTH's research scientists find themselves in this situation every year. The security of employment laws provide legal assurance of an uninterrupted income but not of uninterrupted research in the same field: there is simply not enough research money to assure that.

Money is a problem at all levels of research. At the end of 1978, the NFR asked an international group of physicists to evaluate Swedish experimental nuclear and particle physics. "The first and overwhelming impression", reported the group, "was one of underfunding . . . More precisely, the present materials budgets for research per physicist are too low to guarantee efficient work. They are much lower than current standards internationally, most likely by a factor of two or three."

The group also commented on what it considered the "old fashioned organisation of research at the universities. Younger researchers with international reputations seemed unable to command permanent research positions. The near absence of intermediate tenure positions is disruptive and unhealthy for all long and medium term programmes. The present situation leads to an obsession with the necessity for personal research

programmes with too many subcritical groups."

All this insecurity is having a distinct effect on PhD students, many of whom are increasingly reluctant to finish their doctorates. The PhD student is marginally more secure than the PhD graduate, even if, as UHÄ maintains, some students are forced to earn a living by taking on research not particularly suitable for their doctorates — another contributing factor to the decline in PhD examinations.

Since 1971, the numbers of natural sciences students passing their PhDs has dropped every year; and in 1976/77 it was less than in the mid-1960s even though recruitment to research education has remained fairly constant.

So far, the effects of these problems on research as a whole are not easy to point to. The international evaluation of physics made by the NFR, for example, did not mention any area in which Swedish research in physics is falling behind that in other countries, even though the evaluators often expressed their surprise about personnel and organisational set-ups; and Professor Lindqvist cannot point to any areas in which Swedish scientists are lagging.

But there are problems in sectoral research — research supported by organisations within their own fields of responsibility. According to Professor Erik Arrhenius at the Department of Cellular Toxicology, University of Stockholm, it is very difficult to get PhD students involved in research on the environment. The projects supported by the National Environment Protection Board (NEPB) run for one year at a time, and good scientists want more security than a one-year project can give.

The National Board for Technical Development (STU) has had similar problems with one-year projects. According to Dr Lennart Lindeborg, these problems have triggered the setting up of a new arrangement under which STU will guarantee the researchers' involvement in future projects for five years. The new projects will be in electronics, informatics, speech communication, food production processes, gene technology, the chemistry of wood, enzyme technology, the physics and chemistry of surfaces, fibrous materials and future industrial production systems.

Apart from the plight of present researchers, many are worried about the future. There is a fear that recruitment of graduates for PhD studies will decline. SACO/SR points to the slide in students

completing three years of natural science subjects at universities from 1,390 in 1970/71 to 564 in 1976/77. Bengt Gustafsson maintains that this drop has resulted from the decline in university intake in 1972/73. As the numbers of new students fell off, so did the money for teaching staff; so the universities tried to make themselves more interesting by setting up short courses such as popular astronomy and amateur geology.

These courses have proved almost too successful: students now take them in preference to long courses that can lead to research. Professor Lindqvist thinks there will be a shortfall of PhD recruits in 15 to 20 years. He points to the increasing unpopularity of the natural sciences in secondary schools, saying that it is much easier to earn maximum marks (and it is marks which count for university entrance) in non-science subjects.

There have been piles of papers written about the reorganisation of the universities. Two more commissions will be reporting soon — one on the reorganisation of teaching structures and the other on the education of research students. The first commission is expected to recommend widespread changes to create more tenured research positions and spread the teaching burden more evenly amongst university jobs.

But the crux of the matter is money. Last year, Parliament allocated the UHÄ Sk205 million (about \$46 million) to provide five universities with basic facilities with which to conduct natural sciences research: research education, fundamental research, professorships and appointments for those who had passed their PhDs, secretaries, libraries, instruments and buildings. Engineering research received Sk222 million (about \$50 million), to be spread over six technical universities, for the same sorts of things.

As salaries, equipment and research funds all come out of the same budget, research gets less money as the universities have to go on paying the salaries of scientists whose projects have ended. According to Professor Lindqvist, the University of Lund has to pay more than SK2 million (about \$450,000) a year supporting former docents.

All the relevant authorities are demanding more money, and all the political parties said before last September's election that more money should be given. The Swedish Parliament has in fact agreed that about 1,000 jobs in the public sector — in archives, museums, libraries, positions in planning, commissions of enquiry and some teaching posts at secondary schools — should be given in the first instance to PhDs.

The union also wants the state to give increased support to the development of technology-intensive industries such as telecommunications, energy and defence, to provide secure working places for scientists as well as competitive exports. □

How the popularisation of science narrows the polarisation of the people

In the last of three articles on science and technology in China, **Tong B Tang**, research fellow at Darwin College, Cambridge, UK, reports on scientific education and popularisation

Workers from three provinces of North East China watching a lathe demonstration at a gathering in Shenyang



EDUCATION has always been a central issue in Red China. By comparison, Marxist socialism in the West was also initially strongly associated with an educational movement; it stimulated developments in the philosophy of education, and was instrumental in spreading the concept of education for the working classes. This last influence was never necessary in China: in the past, non-hereditary mandarins were selected by imperial examinations which theoretically anyone could take. Traditionally the populace has been conditioned to appreciate the advantages of education. This deep-rooted exaltation of learning however, makes it all the more important to watch out for intellectual (as well as bureaucratic) elitism.

Two years ago China introduced a nation-wide examination for university entrance to replace nomination at one's place of work. Had everyone equal access to primary education, there would be equal opportunities for all. In practice however, students who can or want to take advantage of further education still tend to come from professional families. Another uneven situation is the formal existence of "key schools", (and universities) which admit "bright" pupils (from the age of five or six onwards) and whose facilities are given priority.

Going straight from high school to university is no longer rare but the long-standing policy of mobilising school leavers to work or settle in the countryside still persists, as affirmed by Chairman Hua in August last year. There are still many technical problems but this policy, besides playing other roles, spreads learning to the rural masses.

Another effective way in which the Chinese try to redress any imbalances in educational equality is their system of marking the university entrance exam. The pass mark is regionally adjusted, and lowered for candidates who have left school and are working in the countryside; ethnic minorities are also given 10 additional marks and educational work in secluded and border areas is paid special attention. Depending on the university (higher for a "key" one), the students need 300-400 out of a possible total of 500 marks to get in. Once there, freshers who come from poorer backgrounds are given extra

tuition.

At present formal degrees are not awarded at the end of the period of study. This will probably change soon: undergraduates sent abroad are reading for degrees. The younger of those scientists sent to participate in research are also encouraged to register for postgraduate degrees; the first Chinese to receive a PhD did so last September, in West Germany. The course structure, originally modelled on the Russian system which emphasises early specialisation, is being modified. Nanjing University, for example, introduced a credit system in 1978 similar to that used in US universities, where the student is freer to choose his or her courses. Summer schools at the pre- or post-doctoral level are also being planned.

At the same time, the Chinese are putting a lot of effort into updating teaching materials: there was a conference on this topic early last year. In most subjects, there are revised editions of former textbooks in circulation, and many universities are now trying them out. (In China most schools use a unified set of texts, while for universities about 70% of the curriculum is standardised by the National Planning Committee in the Ministry of Education.) A selection of course books recommended at major overseas universities is also being looked at. Some of these books may be purchased in bulk when the students are adequately prepared in the relevant foreign languages.

During the past decade most schools and university departments became linked to communes or factories, where pupils and students went periodically to work as part of their education. This scheme has ceased, since their main duty is now assessed to be academic study; so has the "inviting in" of workers and peasants who brought in grass-roots experience and argued with the



Workers in Shanghai examining a water pump design

students and their teachers. However, high-technology pilot plants or small manufacturing units, which provide contacts with workers can still be found in science faculties. More crucially, the long-developed tradition of political work has not disappeared. An academic works for six days a week, one of which is devoted to political study which may include physical labour on the campus. For students in any faculty, there is a 'core curriculum' of philosophy, political economy, Party history and the history of the international communist movement. It accounts for about 200 hours out of a total 2,500 curriculum hours and carries 8 to 10% of overall marks.

With a population of 1,000 million, China has 10 million teachers for 210

million students. Of these, respectively, only 0.2 and 0.9 millions are in the 598 post-secondary institutions. As a medium-term measure to achieve a rapid expansion of tertiary education, an increase has been made in the number of students sent abroad. There are at present 180 post-graduates and 420 undergraduates in North America, Japan and Europe, 80% of whom are in natural sciences. By comparison, 1,300 from the West and the Third World are studying in China. An unusual measure, further, is that a few self-paying students who can afford it are allowed to go abroad; some think that this may be seen to serve the principle of "to each according to his capital".

The opposite tendency, namely, "to each according to his needs", is fostered by a mass movement to raise the scientific and cultural level of the entire nation.

The objectives of science popularisation are not confined to education. It serves to drive out the out-dated ideas in the social consciousness of the whole people, and to generate greater enthusiasm for the Four Modernisations (in agriculture, industry, national defence, and science and technology). The idea is that science popularisation will reduce social polarisation by narrowing the differences between city and countryside, and by reducing the alienation due to the division of labour.

The organisation of popularisation is largely, though not exclusively, in the hands of provincial chapters of the Scientific and Technical Association which are directly responsible to local authorities.

The activities take many forms. In children's and youth clubs, science classes are held and model or instrument building competitions are regularly sponsored. Summer and winter camps teach subjects that range from geology to ecology. Educational exhibitions are shown in the

Scientists and peasants preparing a simplified medium for pollen breeding at the Institute of Genetics of the Academia Sinica



camps too, and in public parks. Public lectures are frequent, sometimes by such leading scientists as Qian Sanqiang whom some foreigners dub the "father of atomic bombs in China".

There are popular science magazines and newspapers on sale throughout the country. No fewer than 1800 popular science books have been published since the beginning of 1978. In August 1979, an Association of Science Popularisation Writers was formed.

Science fiction based on hard science only is expected to flourish soon. Posters of great scientists are constantly in print. I saw the following: Newton, Einstein, Copernicus, Marie Curie, Darwin and the inventor Edison. But disappointingly I didn't find one of an ancient Chinese scientist. Visual arts with scientific themes are also being encouraged. More than 100 new educational films were scheduled last year.

Since May last year "universities of the air" have been started in 29 local TV and radio stations. Correspondence courses are also being extended. In a few years' time there may be twice as many students in these alternative forms of education than in the regular universities and colleges. Full-time schools are also being run to give technical instruction to administrative cadres. Party secretaries and bureau chiefs from the provincial level down and leading personnel in factories, mines and oilfields attend courses lasting two weeks to four months, in farm economics and machinery, stock-breeding, fishery, forestry, enterprise management or computers. It would be amusing for the mandarins in the British Treasury to have to go to evening classes in arithmetic.

It would be unhelpful and unrealistic to imagine that despite their enthusiasm, the Chinese have no major problems ahead. As reiterated in a recent editorial in the *People's Daily*, the question of manpower is the foremost obstacle to the modernisation programme, and to education in particular. Many scientists have little experience of research management: here the Chinese can learn a lot from the West provided they are selective. In the transformation of education is the teachers who constitute the single most crucial sector.

If the development of Chinese science is dependent on politics, then over the next two decades, China might well fulfil its aim of developing a science of its own. It has to be careful to avoid a Scylla of Westernisation and the Charybdis of misguided dogmatism. The Chinese believe that the human activities grouped under the name of science are intimately related to other processes in society — "if society has a technical need, that helps science forward more than ten universities"; at the same time, dialectically the reverse is also true. Science acts on the technico-economic base and is therefore partially self-supporting. □

Friends and foes of science debate in France

Jim Ritter records some observations on what was "effectively the first public debate on science and technology in France for more than a decade". It was staged last month, by *Les Amis de la Terre*.

THERE is a sense of movement about French scientific policy today, a certain loosening of the tight strictures of official silence and lay apathy. What public debate there has been about science has taken place within the cloistered university circles of philosophers and sociologists, while science policy decisions continue to be taken in equal isolation by scientific and political mandarins.

Hence the strangeness to the Anglo-Saxon eye of the programme for a debate on science and technology policy organised by *Les Amis de la Terre* and held in Paris one weekend last month. There was little discussion of concrete points of technology — nuclear power safety, pollution, and so on. There was much of more epistemological questions — the nature of quantum mechanics, the question of systems analysis. But the main stress was biological; a reaction to the "biological determinism" now being vigorously propagated in France by a group of influential far-Right intellectuals (the New Right) who claim that modern biology has "proven" the inequality of races, sexes, and classes. The organisers did well in assembling, for virtually the first time in a decade, a number of top administrators, active researchers, authors and journalists to face a concerned public.

The debate started with a panel discussion among three physicist-philosophers on the interpretation of quantum mechanics. The hall was surprisingly full and the question of whether the Copenhagen interpretation was idealist or not was entered into with great gusto. But the debate was inconclusive, the question remaining rather elusive though the level of debate was one which, in the UK, would only be found in specialised seminars.

The afternoon session on bioengineering was livelier and more familiar. The panel consisted of three directors of biological research and two young researchers. The difference of opinion ran along the same line, the mandarins seeing no reason why genetic manipulation should not be prosecuted with all possible speed while the two opponents pointed out what they saw as disquieting experimental evidence and questioned the point of research. The audience of more than 100 readily joined in. They were clearly worried while the administrators were equally clearly unused to hostile questions and overreacted. The

scientists quoted particular experiments to support their claim that genetic manipulation was/was not dangerous but never seemed to come to grips either with each other's claims or with the general unease among the lay members of the audience.

Sociobiology, in the next session, generated a more unanimous attitude, with two of the three biologists on the panel having written recent popular books on the subject. A clear presentation of the basics of the theory, together with an uncovering of the political attitudes underlying its "objective, scientific" attack on women, non-whites, and the poor, led to one of the liveliest debates of the weekend. The discussion was continued into Saturday evening with further analysis of the particular use of women as a target by the New Right.

Sunday morning and it was the turn of systems analysis. Here both the panel and the audience were divided on the merits of a general systems approach. Some saw it as the answer, some would replace it with catastrophe theory, some saw any such search for a totalising theory which explains all levels of the universe at once as obfuscation. The search for and critique of panaceas went on into the following physics debate. Are microprocessors the solution or the problem? Will larger and larger particle accelerators advance physics as much as more modest investments elsewhere?

The final session was on social responsibility and the hall was packed to overflowing, the debate heated. The presence of trade union representatives and members of various political and environmental groups made this discussion the most familiar to the Anglo-American ear. But when one participant pointed out that "we've heard this all before, ten years ago", one was reminded of just how damaging the long silence in French public debate has been.

In any case the science policy debate has reopened in France after a long hiatus. Any facile optimism about how easy it would be to simply confront the "expert" and "the public" has evaporated after 20 hours of long and arduous discussion. For when two "experts" disagree, how is one to choose? And how can one pose the right questions without already knowing some of the answer? But *Les Amis de la Terre* are pleased with it and plan others for the future. □

NEWS AND VIEWS

Antarctic ice surges and theories of glaciation

from D.Q. Bowen

THEORIES of ice ages are never far from the limelight, but with the demonstration that the Earth's climate over the past 500,000 years has followed changes in the amount of solar radiation received at certain latitudes, as predicted by the Milankovitch model (Hays *et al.* *Science* **194**, 1121; 1976), the mystery seemed to be solved. But those authors would be among the first to admit that the model is really no more than a black box; a celestial mechanical input is followed by the output of a climatic signal. Although past behaviour of the climate can be deduced from oxygen isotope analyses of deep-sea cores, which allow inferences to be drawn concerning the interaction of the Earth's atmosphere, oceans and cryosphere, exactly how an ice age starts remains unknown. Any model should also explain not only how glaciation is initiated but also how it is brought to an end.

In 1964, A.T. Wilson (*Nature* **201**, 147) suggested that because the Antarctic ice-sheet is inherently unstable it would surge periodically into the southern ocean and form a huge ice-shelf. This would increase the Earth's albedo and the transfer of the consequent cooling to the northern hemisphere would lead to glaciation. Because the ice-shelf could not be sustained its eventual break-up would decrease albedo, heat would be transferred to the northern hemisphere and glaciation terminated. This was subsequently modelled and deemed eminently feasible climatologically (Flohn *Quaternary Res.* **12**, 135; 1979).

J.T. Hollin (*Nature* **208**, 12; 1965) pointed out that the global rise in sea-level after such a surge, possibly amounting to 15 m, should be capable of geological verification: that is, by evidence of a rapid, though short-lived, rise in sea-level at the end of interglacials. In 1977 (*Boreas* **6**, 33), he argued that it occurred at the end of oxygen isotope stage 5e, to initiate the Last

Glaciation; but on page 649 of this issue of *Nature* he now suggests it was later, at the end of stage 5c. How is this shown? Unequivocal geological proof for such sea-level rises, and inferred surges of Antarctic ice, is difficult to obtain. How can a raised marine deposit be ascribed to such an event? In south-east England Hollin was able to show that some marine transgressions took place late in interglacials from the fact that their beach deposits overlie terrestrial ones dated by pollen analysis. Generally however, the rapidity of the postulated surge in sea-level, its brevity, and the incompleteness of the stratigraphic record, have conspired against a full and convincing demonstration. Nor is the surge likely to be recorded in the deep-sea cores, with their comparatively slow sedimentation rates, for it would have gone in less than 2,000 years — possibly considerably less.

Important new evidence bearing on the Wilson hypothesis comes from the Huon Peninsula, New Guinea, where a flight of uplifted coral terraces occurs more or less on the boundary between the West Pacific and Australian lithospheric plates. The old orthodoxy, which directed sea-level studies to stable coastlines (where, unfortunately, repetitive high sea-levels would have destroyed most pre-existing evidence), should now be received with caution, for the evidence is more effectively preserved in tectonically-rising areas. Provided that the tectonic factor can be separated from the depression caused in land level by the glacial masses of ice, which has been accomplished with considerable success in New Guinea (Chappell *Bull. Geol. Soc. Am.* **85**, 553; 1974) detailed records of sea levels are available at least for the past 130,000 years. Furthermore, the palaeoecology of the corals allows precise inferences to be drawn about rises and falls in sea-level. Unaltered aragonite coral may also be dated by $^{230}\text{Th}/^{234}\text{U}$ determinations. Thus much of the required critical data to test for sea-level surges is available.

P. Aharon, J. Chappell and W. Compston (page 669, this issue of *Nature*) show that the record is sufficiently good to establish, and date, sea-level fluctuations between 140,000 and 105,000 years ago. Stable isotope work on shells of the giant clam *Tridacna gigas* also provides information on the oxygen isotopic composition of the global ocean during this period. Because the clam lives in isotopic equilibrium with the ocean, a feature of some significance is an anomaly 120,000 years ago — during the Last Interglacial. This anomaly is interpreted as showing that water temperatures 120,000 years ago were cooler than at 130,000 years. Moreover the cooling took place within 1,000 and 2,000 years during a rise in sea-level of 8 m. Aharon *et al.* attribute this event to an Antarctic surge which caused sea-level to rise and transmitted cold water northwards. Thus the required geological test for Wilson's theory appears fulfilled. But did this event, 120,000 years ago, or the similar one identified by Hollin at ~95,000 years ago, initiate the Last Glaciation?

Evidence submitted in both papers is convincing; both use the relatively objective stratigraphic framework provided by the deep-sea oxygen isotope record. This, however, is concerned with chronostratigraphic 'Stages' — time-intervals defined from actual lithostratigraphic units (Shackleton & Opdyke *Quaternary Res.* **3**, 39; 1973) — and not with wholly inferred climatostratigraphic concepts such as 'glacials' and 'interglacials', or 'stadials' and 'interstadials'. It is quite possible that Antarctic surges occurred on several occasions not always related to interglacials as presently, and loosely, defined. In the period under consideration, a further difficulty arises from lack of agreement on how the end of the Last Interglacial should be defined. The classical Last Interglacial (Eemian) ended 120,000 years ago, but Woillard (*Quaternary Res.* **9**, 1; 1978) showed

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that two further interglacials occurred before the Last Glaciation commenced, 70,000 years ago. Definition rests on the classical north-west European pollen analytical demonstration of deciduous forest woodland. This somewhat parochial classification has been generally and uncritically accepted everywhere.

This vexed question is not new; volume 2 (No. 3) of *Quaternary Research* (1972), was devoted to the end of the 'Last Interglacial'. It is clearly desirable to agree on the definition of Global Standard Interglacials in relation to Stages objectively

defined. In terms of the two contributions in this issue, neither of the coolings which their respective authors attribute to Antarctic surges led directly to the maximum build-up of ice during the 'Last Glaciation'. It is not impossible that such surges were the rule rather than the exception and that many abrupt temperature falls in the Pleistocene record are to be so explained. Perhaps major periods of continental glaciation only took place when a combination of Antarctic surge and favourable Milankovitch conditions arose. □

Osteology and prehistoric society

from Stephen Shennan

RECENT work on the chemical analysis of human bone from archaeological contexts confirms and extends the potential of human osteological studies for throwing light on man's social and economic development.

Archaeologists are increasingly concerned with explaining cultural change in terms of the social and economic processes at work within and between societies. One important advance has been in the use of evidence from excavated cemeteries to reconstruct past social organisation. Quantitative studies of the grave goods of dead individuals and the form and degree of elaboration of their burial monuments have been used to reconstruct the development of increasingly complex and differentiated societies as well as, in some cases, their devolution to more simple forms (for example Peebles *Mem. Soc. Amer. Archaeol.* 25, 68; 1971; Rothschild, *Amer. Antiquity* 44, 658; 1979).

Remarkably little attention has been given to the body itself however. Apart from its sex and age at death archaeologists have shown little interest in the information to be gained from the skeleton, and the obligatory physical anthropologist's report usually remains an unintegrated appendix. However, an increasing number of studies, particularly from North America, shows that the skeleton can yield information on aspects of diet and biological stress in past populations which are otherwise inaccessible and are vital to an understanding of cultural change.

In a recent paper Lambert, Szpunar and Buikstra (*Archaeometry* 21, 115; 1979) describe the results of a chemical study of the skeletons of two burial populations from the eastern United States. The first belongs to the Middle Woodland period, about AD 400, when hunting and gathering provided a great variety of foods. The second belongs to the Late Woodland

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period, about AD 1200, when maize farming is well documented in the area. The bones were analysed by atomic absorption spectroscopy after sample preparation by a new method involving virtually complete dissolution of the bone ash. Particular attention was given to the problem of contamination of the bone by the surrounding soil and the conclusion was reached that although concentrations of iron, aluminium, manganese and potassium may largely reflect soil contamination, strontium, zinc, calcium, sodium and probably copper and magnesium give analyses that are representative of the bone.

The authors were able to investigate differences within as well as between each population. In the earlier one males and females had statistically identical bone compositions for every element examined, whereas in the later one there were significant differences, males having lower strontium and calcium and higher zinc concentrations, all diet-related elements. It seems that dietary differences between males and females developed which it is tempting to relate to the development of maize cultivation, and which may be associated with a greater differentiation of sex roles; the lower strontium concentration for males is at least consistent with a higher protein diet. Equally interesting, at the earlier site, where archaeological information indicated that the population could be divided into three groups of differing rank, it seemed that while the upper two groups did not differ significantly from one another in any elements, there were significant differences between the lowest group and a combination of the upper two in strontium, zinc, magnesium and sodium, again diet-related. Here the archaeological and analytical results together can show how the social distinctions marked in the burial rites correspond to dietary differences in life, although the nature of these differences is not yet clear.

In this example the need of the archaeologist for the physical anthropologist and chemist is clear enough but Buikstra (*op. cit.*) has also shown how the anthropologist can be misled if he fails to take archaeological considerations into account. The high frequency of pathologies in certain North American skeletal series from habitation sites of the Mid-Archaic period, has been ascribed to the rugged conditions of life. However, Buikstra was able to show that this phenomenon was a result of the nature of the sample: normal adults were buried in separate cemeteries but individuals not able to perform the range of 'normal' adult tasks were buried in the occupation area.

The potential of integrated work is further illustrated in the recent study by J. Lallo and J. Rose of another two prehistoric populations from eastern North America (*J. hum. Evol.* 8, 323; 1979). They selected two populations from a single site in a period for which there is strong archaeological evidence of social and economic change, the first belonging to the Mississippian Acculturated Late Woodland period, AD 1050–1200, and the second to the Middle Mississippian, AD 1200–1300. During this time society became increasingly less egalitarian, contacts became more widespread, and subsistence came to be more dependent on maize. Infectious lesions in bone were twice as common in the later period, as well as being more severe. Porotic hyperostosis, a condition of the bones of the skull reflecting increased bone marrow activity, was also more common. This condition has been observed elsewhere in prehistoric maize-eating groups (El Najar *et al. Amer. J. phys. Anthropol.* 44, 477; 1976) and interpreted as reflecting iron deficiency. Various dental defects reflecting nutritional stress were also more common in the later period. Finally, examination of the distribution of age at death in the two populations showed a decrease in the life expectancy from 33 to 24.3 years. Lallo and Rose go on to offer a systematic explanation of these phenomena, indicating the way in which the social and economic changes independently documented lead to increasing disease and nutritional stress.

One hypothesis of this type has been put forward recently by R. Santley and E. Rose for the Basin of Mexico, one of the cradles of Mesoamerican civilisation (*World Archaeol.* 11, 185; 1979). They argue that between 1500 BC and the Spanish conquest major periods of population growth correlated with periods of balanced nutrition, whereas phases of population stability corresponded to times of nutritional shortages and stress. They suggest that in hard times the elite urban populations would have used their power to ensure an adequate nutritional base for themselves at the expense of the rural population. Whether or not their idea proves correct, its testing will bring

osteological analysis of the type described above into the explanation of some of the major developments in New World prehistory.

All these studies show the importance of using the osteological evidence to make inferences about the human biological changes which have accompanied social and economic development, and which not only tell us more about those developments but may themselves have been responsible for them in certain circumstances, providing one of the 'kicks' which set positive feedback processes in motion. □

Sails set for the myosin active site

from Peter Chantler

WHEREAS kinetic secrets are being prized almost daily from the myosin and actomyosin ATPase mechanisms knowledge of their structural aspects remains in the dark ages. The quest for structural information on the active site of myosin has been bedevilled by false leads. One example is that of the two thiol groups (SH-1 and SH-2) on each myosin head which were originally considered essential to the myosin ATPase mechanism, possibly being intimately involved with nucleotide at the active site. More recent studies however, have thrown doubt on the idea that these thiol groups are directly involved in the active site and a new twist to the story has recently emerged from Yount's laboratory at Washington State University. By an innovative technique in which the fate of radiolabelled nucleotide substrates was followed during chemical modification of myosin with cobalt, they have clarified some aspects of the relation of the thiol groups to the true active site and at last opened up a way for its unambiguous definition (Wells *et al. Biochemistry* 18, 4793; 4800; 1979; Wells & Yount *Proc. natn. Acad. Sci. U.S.A.* 76, 4966; 1979).

Taking advantage of the exchange characteristics of Co (II) and Co (III) coordination complexes, myosin subfragment 1 (S-1) was exposed to a mixture of CoCl_2 , 1,10 phenanthroline and $[\text{Co}^{\text{III}}(\text{phen})_2\text{CO}_3]^+$ with a resultant inactivation of the ATPase activity (Wells *et al. Biochemistry op. cit.*) caused by tight chelation of one cobalt ion per mole S-1 which occurred in parallel with the loss of two sulphhydryl groups. The mechanism proceeds by way of a $\text{S-1.Co}^{\text{II}}(\text{phen})_x$ intermediate (Co^{II} freely exchangeable) which undergoes exchange with $[\text{Co}^{\text{III}}(\text{phen})_2\text{CO}_3]^+$ to yield a $\text{S-1.Co}^{\text{III}}(\text{phen})_x$ compound (Co^{III} not exchangeable). Such *in situ* oxidation of the bound

Human teratocarcinoma workshop

Clinicians and mammalian embryologists are launching a concerted attack on human teratocarcinoma. The embryologists are moving on to this new challenge after some years spent manipulating mouse teratocarcinoma stem cell lines, obtained originally from tumours of the murine gonads. The value of such cell lines lies in the ease with which they can be induced to differentiate in a manner thought to be analogous to certain events of natural development. Although mouse development could hardly be said to be understood yet, insights into human development are now hoped for.

But embryologists are faced with the usual problems of working with human material. It is difficult to obtain, requiring the cooperation of surgeons who will ensure that a reasonable specimen is put aside before the whole tumour is claimed by a pathologist and doused in formalin. The problems of

pathology and nomenclature are also formidable, and participants at a European workshop on human teratocarcinoma at Imperial Cancer Research Fund Laboratories (ICRF) in London on 28 January were not always sure what they were talking about. Nor were they all particularly confident of the difference between teratocarcinoma, seminoma and yolk sac tumour, although all three originate in the same anatomical region.

In future they will cooperate in obtaining cell lines and in characterising those already established. The workshop will gather again in the autumn. Meanwhile information about the cell lines, their biochemical and immunological characteristics, is to be collected by Brigid Hogan of ICRF's Mill Hill laboratories for subsequent distribution. Later there may be a human teratocarcinoma newsletter.

M.L.

cobalt is extremely mild; indeed, the $\text{S-1.Co}^{\text{III}}(\text{phen})_x$ can be reduced by short incubation with reagents such as $\text{Fe}^{\text{II}}\text{EDTA}$ which fully restore enzymic activity. The simultaneous loss of $\text{NH}_4^+ - \text{EDTA}$, Ca^{2+} and $\text{Mg}^{2+} - \text{ATPase}$ activities on cobalt binding and the blocking of the binding by *p*-N,N'-phenylenediamine (which crosslinks SH-1 to SH-2) identified the two sulphhydryls lost as SH-1 and SH-2 and showed that these two thiols could be cross-linked by the cobalt and therefore approach to within 3-5 Å of each other (the distance depending on whether there was *cis* or *trans* chelation).

When the fate of ^{14}C -labelled nucleotides was followed during the course of Co modification, inactivation of the ATPase amazingly paralleled incorporation of nucleotide so that complete inactivation occurred on simultaneous binding of one gram-atom of cobalt and one mole of nucleotide per mole S-1. This not only directly demonstrates the separate identities of the active site and the SH-1 and SH-2 thiols but also shows, consistent with other evidence, the interdependence of the thiol and active sites. When cobalt chelates SH-1 and SH-2, nucleotide at the active site is prevented from leaving; when nucleotide binds to the active site, cobalt chelates SH-1 and SH-2 much more easily. In addition, a new phrase enters the textbooks: active site trapping — a technique, in serendipitous cases, as here, of great potential power. When the experiment was performed in the presence of MgATP the trapped nucleotide always manifested itself as MgADP . Although the relationship between cobalt modification and nucleotide hydrolysis is unclear it is apparent from the relative rates of the two reactions that the trapped ADP did not originate from a rebinding of previously hydrolysed substrate.

As active site trapping seems to be equally effective in the presence of adenosine 5'-(β,γ -imido)-triphosphate, a non-hydrolysable analogue of ATP, the technique may well offer some insight into mechanism. But its real future lies in conjunction with photoaffinity probes such as 8-azidoadenosine triphosphate which the authors have also trapped (Wells & Yount *op. cit.*). The power of the photoaffinity probe, in more conventional mode, has been seen recently in the binding and incorporation of arylazido- β -alanine-ATP into proteolytic subfragments of myosin followed by tryptic proteolysis (Szilagyi *et al. Biochem. biophys. Res. Commun.* 87, 936; 1979). The reagent labelled an N-terminal 25K peptide of myosin; there was no incorporation into the fragment containing SH-1 and SH-2. As this latter fragment is separated from the 25K peptide by a 50K peptide (Szilagyi *et al. op. cit.*) it is apparent that the sequentially remote 'essential' thiol region must be juxtaposed near to the active site in the tertiary structure of the enzyme.

Detailed speculations on which amino acids are involved at the active site of myosin are unwarranted but the most likely residue seems to be arginine (Mornet *et al. Eur. J. Biochem.* 100, 421; 1979), found at the heart of many nucleotide-binding enzymes (Riordan *et al. Science* 195, 884; 1977). Tryptophan, on the cards for many years because of the nucleotide-dependent tryptophan fluorescence enhancement of skeletal muscle myosin (Werber *et al. Biochemistry*, 11, 2872; 1972) seems less likely now in view of the absence of such effects in invertebrate myosins (Chantler & Szent-Gyorgyi *Biochemistry* 17, 5440; 1978). Lysine, histidine and tyrosine have also been proposed. At last the opportunity to find out for certain is here. □

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Ribonuclease still under attack

from Barry Robson

THE enzyme ribonuclease has always been popular with experimentalists seeking to understand the relations between chemical formulae, three-dimensional structures, and functions of protein molecules. Ribonuclease is a particularly attractive weak spot in nature's defences because it was the first enzyme to have its chemical formula determined (as a sequence of amino acid residues), one of the earliest enzymes to have its three-dimensional structure elucidated, and the first protein for which it was carefully demonstrated that the chemical formula alone determines the three-dimensional structure and function. It continues to be a prime target in a number of recent investigations.

One important approach to understanding the relationship between the chemical formula and the final structure is to attempt to follow how a protein molecule folds up after it has been unfolded. In particular, it would be of interest to know how a protein folds up so rapidly: the current theory is that it is rapidly directed towards the native structure by a process of nucleation in which certain structural features such as α -helices and β -pleated sheets form first, propagating further structure around them. By tritiumating the exchangeable amide protons of unfolded RNase A and following exchange of tritium with the surrounding solvent during refolding, Schmid and Baldwin (*J. molec. Biol.* **135**, 199; 1979) have been able to confirm that the protein does fold up by a stepwise process, early stages being hydrogen bonded structures in which the tritium is protected from exchange. Since α -helices and β -pleated sheets are such structures, this would seem at first consideration to support the nucleation model. In particular, it supports the idea that such intermediate structures are metastable, possibly in appropriate conditions stable, and form relatively rapidly, as suggested previously by studies on penicillinase using more standard techniques (Robson & Pain *Biochem. J.* **155**, 325; 1976).

However, the nucleation idea is complicated by the experimental observations that the stability of the intermediate structure depends on interactions with the rest of the protein. In their case Schmid and Baldwin find that an artificially cleaved-off fragment of ribonuclease known as the S-peptide seems to be essential for the stability of the intermediates. In a further detailed study

Labhardt and Baldwin (*J. molec. Biol.* **135**, 231 and 245; 1979) observed (1) that S protein (ribonuclease A without S-peptide) folds up some hundred times more slowly than intact ribonuclease A, and (2) that on adding S-peptide to S-protein, the combination of S-peptide and S-protein occurred at an intermediate stage, rather than at the end of the folding process. Labhardt and Baldwin exclude the model for protein folding in which there is an initial slow diffusion of the molecular conformation into a restricted range of three-dimensional structures, and demonstrate instead successive folding stages with rates dependent on the stabilities of intermediates from preceding steps. Whether this supports or excludes the classical model for nucleation depends on how one interprets that model. Rightly or wrongly, many workers seem to associate the idea of nucleation with a key directing step, the question of rates being one of their own preference. That Labhardt and Baldwin are more proper about the definition of nucleation is, however, irrelevant, since they provide a self-consistent and meaningful description of the folding process.

Baldwin and colleagues also give considerable attention to the fast and slow folding species of RNase in their studies. In this they accept the current interpretation that the slower species is the product of a kind of isomerisation of proline residues in the unfolded form. This may, however, be a case in which attention to one enzyme is less satisfactory. The interpretation in terms of isomerisation is best tested by reference to the different rates of folding kinetics of many different proteins, correlating the proline content with those rates (Stellwagen *J. molec. Biol.* **135**, 217; 1979).

Studies of this type provide a logical framework for the theoreticians, whose principal goal is to predict the three-dimensional structure of a protein from its amino acid sequence alone, and ultimately to design new proteins by choosing the amino acid sequence to give the required conformation and function. A major difficulty here is that many amino acid residues considered alone have only a subtle effect on the overall conformation of the molecule: all the amino acid residues tend to interact in a complex way. The theoretician is faced with having to handle calculations concerning a delicate balance of forces and it is doubtful whether his armoury is as yet adequate to this task. Gutte, Daumingen and Wittscheiber (*Nature* **281**, 650; 1979) recently mounted an audacious rear attack on this problem by designing an artificial RNase of 34 residues, having chosen amino acid residues and their sequence which would seem likely to be a very strong generator of the conformation required. Particular emphasis was given to the strong tendencies of certain residues to form α -helix or β -pleated sheet. The philosophy

was that the more subtle and ambiguous aspects should be avoided, and this rationale seems justified by their subsequent synthesis and investigation of the protein. Briefly, although a detailed analysis of the three-dimensional structure was not possible, the protein indeed had significant activity, particularly when in a dimerised form.

More precisely, one should say that the production of RNase activity may have been something of a bonus, since they state that their immediate aim was to produce a protein with nucleic acid binding ability. This is a necessary but not sufficient condition for RNase activity and it is conceivable that catalytic activity might have to have been introduced at a later stage by further refinement of the sequence. This turned out not to be the case, and it seems that their work brings the status of the theoretician's art one step nearer to practical application. However, noting that the activity of the artificial RNase was only a few per cent of natural RNase, one should consider appropriate control experiments. As Gutte *et al.* discussed, random amino acid sequences often have significant enzymic activities of various types. Perhaps it would have been helpful to compare the activity of their artificial RNase with that of random amino acid sequences of similar length and composition to establish the importance of specific consequences. If the histidine residue was responsible for the activity, then the precise environment may have been rather less important. Further, catalytic activity of another kind has been associated with a mere 10-residue sequence (Chakravarty *et al. Experientia* **29**, 786; 1973), so it does not follow that all of the artificial molecule was in the expected conformation. Particularly when the substrate is a macromolecule, this may itself be a source of the appropriate environment and conformational stability, involving a kind of 'induced fit' of the synthetic protein.

RNase and their relatives are among some of the smallest enzymes known and their ability to interact with nucleic acid makes them useful models both for modern and prebiotic systems of more general interest. Undoubtedly the recent studies will encourage the continued use of RNase and its synthetic analogues. □

Correction

In the article 'Probing the insulin receptor' (*News & Views* **282**, 11; 79) the incorrect impression may have inadvertently been given that the photolabelled probes used by C.C. Yip *et al.* were less well characterised than those of other groups. (line 4, paragraph 3, page 11). The probes are in fact specifically photolabelled at the B-29, A1-B29 or B1 positions, which was not stated in the article.

Post-receptoral processes in colour vision

from J.D. Mollon

ALTHOUGH our subjective experience of hue may seem endlessly varied, the process of colour vision begins with just three classes of retinal receptor, each sensitive to a large part of the visible spectrum. Long before these delicate structures could be directly measured by physiologists, the limitation to three parallel channels was deduced by psychophysicists — those who work out the logic of sensory systems from the responses of conscious observers to systematically varied inputs. More difficult for the psychophysicist was the task of discovering how absorption varied with wavelength for the individual classes of receptor, since these 'spectral sensitivities' overlap substantially and it is not possible to isolate, say, the short-wavelength receptors simply by stimulating the retina with violet light. However, experimental stratagems were developed to cause an observer's response to depend on signals from only one class of receptor: for example, the psychophysicist might use monochromatic adapting stimuli to suppress two of the three classes or he might choose spatial and temporal parameters for his target stimulus that were suspected to favour one class of receptor or he might seek observers genetically lacking either one or two of the three classes. For many decades, psychophysical estimates of the receptor sensitivities differed widely, but the past 20 years have seen a quiet convergence on the wavelengths 440 nm (violet), 535 nm (green) and 565 nm (yellow green) for the peak sensitivities of the three classes; and although these values need correction for selective absorption in the media of the eye and although second-order disagreements remain, the psychophysical estimates are in first-order agreement with very recent physical measurements of individual receptors (see papers by Bowmaker and collaborators in *J. Physiol. Lond.*, 298, 1980).

Isolating opponent channels

It is generally held that a second kind of parallelism follows the receptor stage. There are some channels (loosely called 'luminance' or 'non-opponent' channels and probably needing further subdivision) that receive signals of the same sign from the receptors with peak sensitivities at 535 and 565 nm, and there are others (the 'colour-opponent' channels) that receive antagonistic signals from different classes of receptor. The colour-opponent channels extract information about wavelength by comparing the relative absorption in different classes of receptor. (The receptors themselves, of course, are individually colour-blind, since they are broadly tuned and since a photon, once

absorbed, yields the same signal whatever the wavelength of the stimulating light.)

Psychophysicists have developed stratagems to isolate individual channels at this second stage and several experimental techniques for isolating opponent channels became explicit at a meeting on post-receptoral processes held in London on 9 January by The Colour Group, an interdisciplinary society linking those with physical, physiological, and aesthetic approaches to colour.

If detection thresholds are measured for weak monochromatic stimuli presented on various steady adapting fields, detection by opponent channels is favoured if the targets are of long duration or large area — presumably because these channels integrate in time and space more than do (some) non-opponent channels. Evidence for this position was presented at the meeting by E. King-Smith (University of Manchester) and by D.H. Foster (University of Keele).

King-Smith suggested that strong, white-adapting fields will adapt non-opponent more than opponent channels, because the sensitivity of the latter is controlled by the difference in signals from different receptors and so will change little with absolute light intensity. J. Mollon (Cambridge University) added the complementary suggestion that strongly coloured, monochromatic fields (as used classically by Stiles) cause detection to depend largely on non-opponent channels, because such fields disproportionately desensitize opponent channels.

Foster described a new technique for isolating opponent channels. On a standard adapting field is superimposed a small, steady 'auxiliary' field, which is congruent with the target flash. If, say, the target is of long wavelength and only the main adapting field is present, the field is most effective in reducing sensitivity when its wavelength is ~570 nm; introduction of the auxiliary field shifts the wavelength of maximal field sensitivity to ~610 nm, suggesting that detection now depends on a channel which receives excitatory signals from the 565-nm receptors and antagonistic signals from the 535-nm receptors. The effect of the auxiliary field is abolished if the auxiliary field is significantly larger than the target spot. Presumably the presence of a contour coincident with that of the target masks a spatial transient that would otherwise allow detection by a non-opponent channel.

Mollon proposed that detection will depend only on an opponent channel if the input signal is confined to the violet-sensitive receptors. Perhaps because the short-wavelength component of the retinal image is usually out of focus (owing to the chromatic aberration of the eye), signals from the violet-sensitive receptors seem to

be used only to provide information about hue. Because these receptors are far removed in peak sensitivity from the other two classes, the sensitivity of the opponent channel can be modulated with long-wavelength fields without significantly changing the rate of photon absorptions in the violet-sensitive receptors themselves — a great experimental convenience.

According to a hypothesis of Pugh and Mollon (*Vision Res* 19,293; 1979), an opponent channel is most sensitive to input perturbations when it is near the middle of its operating range. In collaboration with P.G. Polden and A. Stockman, Mollon has found large oscillations in sensitivity to violet targets during adaptation to a bright yellow field; these are thought to arise from changes in the balance of inputs to the opponent channel during bleaching of the long-wavelength receptors. King-Smith reported that in several ocular diseases (for example, retrobulbar neuritis and glaucoma) patients show a greater loss of sensitivity to short-wavelength targets when the eye is adapted to a yellow field than when the adapting field is white and relating this finding to the above hypothesis proposes that the operating range of opponent channels is reduced in disease.

It is well established that adaptation to a visually presented grating will briefly raise the threshold for a subsequently presented 'test' grating of similar orientation and spatial frequency. K. Ruddock (Imperial College, London) has previously reported that if the adapting and test gratings are presented to one eye, a low-frequency spatial pattern presented to the other eye during adaptation will reduce the extent of adaptation. V. Waterfield and he have now shown that the effectiveness of this contralateral stimulus depends on its wavelength. Moreover, variation of its actual pattern intriguingly reveals channels of different spectral sensitivities: thus a simple grating gives the photopic luminosity function, a pattern of blobs reveals a long-wavelength channel with opponent inputs from the 565-nm and 535-nm receptors, and the negative of the latter pattern gives a green-sensitive mechanism with a small inhibitory long-wavelength input.

Two possible pitfalls

Many papers in the recent literature have advanced a further stratagem for isolating colour-opponent channels: the observer is required to detect a spatial or temporal transition between stimuli that are of different wavelength but equal luminance. However, I feel that it is unlikely that this device will safely eliminate detection by 'luminance' channels. Suppose that the target is a red field briefly substituted for a white field of equal luminance. At the onset of the red field, an increment is presented to the 565-nm receptors and a decrement to the 535-nm receptors. We are

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obliged to adopt an assumption for which there is no proof, that the transient responses generated in the two classes of receptor will exactly cancel out in all the putative 'luminance' channels; we must suppose that the weightings of inputs from the different receptors are in a constant ratio for all channels and that the transient waveforms of different signs are symmetrical. Transients in space and time are biologically significant and it would be odd if the retina allowed them to be lost in this way.

A second pitfall may lie in relating the detection measurements mentioned above directly to the classical phenomenological measurements (see for example Hurvich & Jameson *Psychol. Rev.* 64, 384; 1957) that kept alive the concept of opponent processes during a period of neglect. General understanding of the first stage of colour vision was earlier held back by a widespread but unjustified belief that the peak sensitivities of the three receptors should correspond to phenomenologically primary colours, red, green and blue; it may equally be a mistake to suppose now that the opponent processes of the retina must be directly related to the phenomenological oppositions of blue and yellow and of red and green. S. Zeki (University College, London) presented his measurements of the spectral sensitivities of individual nerve cells in Area V4 of the cerebral cortex of the rhesus monkey; even at this central level, although many cells have narrow bandwidths (10–20 nm), the peak sensitivities do not cluster very tightly at the four phenomenologically pure hues, blue, yellow, green and red, but are distributed throughout the spectrum, with possibly a gap at 560–570 nm.

Colour constancy

As we view an object from different distances and angles or under different illuminations, our perception changes much less than does our retinal image. This stability of our perception is known as perceptual constancy. It is an everyday observation that a sheet of white paper looks equally white in daylight and in the yellowish illumination of domestic tungsten light, although if outdoor film were used to photograph the paper indoors, the resulting transparency would have a strong yellow cast. The correction achieved by our visual system (but not by the photographic process) is called colour constancy. One of the most interesting results presented at the meeting was Zeki's finding that some individual cortical cells show colour constancy: if an array consisting of many different coloured patches is placed before a monkey, and, say, a green patch is arranged to cover the receptive field of a green-sensitive cell, the cell responds to the patch despite gross changes in the spectral composition of the illumination falling on the array; but the cell does not respond if, say, a red patch is placed in its receptive field and the overall illumination

is adjusted so that the spectral composition of the flux reflected from the red patch was identical to the flux previously reflected from a green patch to which it did respond (Zeki *Nature* in the press). Under the latter condition, of course, the patch looks red, not green, to a human observer (see *Land Sci. Amer.* 237, December; 1977) just as, in the classical demonstration of size constancy scaling, an after-image of fixed retinal dimensions looks of different sizes when projected on surfaces at different distances.

Colour preferences

The layman reasonably expects the colour scientist to be much concerned with people's colour preferences, but the experimental study of colour preference has an unhappy history of inconsistent results and has been neglected in recent years. Reviewing this field for the meeting, I.C. McManus (Bedford College and St. Mary's Hospital, London) noted that earlier

studies had confounded variation in hue with variations in saturation and lightness, and he described a study, performed in Cambridge in collaboration with A. Jones and J. Cottrell, in which this failure was corrected. Another feature of this new study was the use of the 'method of paired comparisons': each of a large set of coloured chips was paired with every other chip and the subject was asked to express a preference between the members of each possible pair, thus allowing the experimenter to derive a measure of internal consistency. When saturation and lightness are held constant, the population shows a clear preference for blue and a clear dislike of yellow. Further analysis shows differences between individuals but consistency within an individual's data; differences between individuals seem to be almost entirely due to differences in lightness preference and saturation preference, with very small inter-subject differences in hue preference. □



100 years ago

THE CRAYFISH¹

Common and lowly as most may think the crayfish, it is yet so full of wonders that the greatest naturalist may be puzzled to give a clear account of it." These words from von Rosenhof, who in 1755 contributed his share to our knowledge of the animal in question, are cited by Prof. Huxley in the preface to the careful account of the English crayfish

¹"The Crayfish: an Introduction to the Study of Zoology." By T.H. Huxley, F.R.S. (London: Kegan Paul, 1880.)

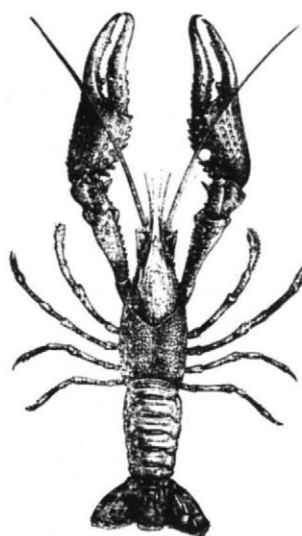


FIG. 4.

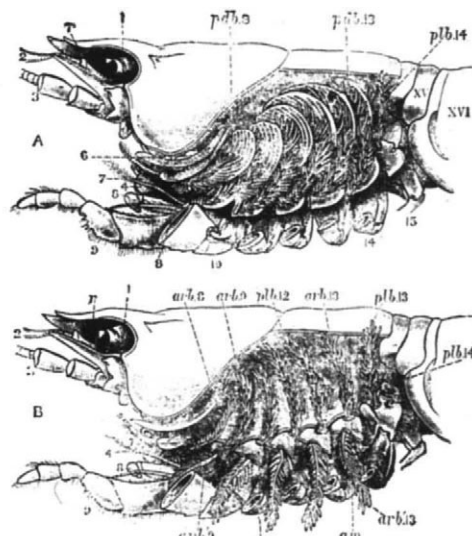


FIG. 5.

From *Nature* 21, 12 Feb., 353; 1880.

REVIEW ARTICLE

Cellular site of opiate dependence

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Experiments on whole animals, guinea pig isolated ileum, individual neurones in situ and cultured neuroblastoma–glioma hybrid cells indicate that opiate dependence and associated tolerance develop within neurones bearing specific opiate receptors (opiate-sensitive neurones). The essential change appears to be an hypertrophy of the cyclic AMP system, in response to inhibition by opiate of a neuronal adenylate cyclase.

CHRONIC treatment, either continuous or intermittent, of a mammal with an opiate drug produces tolerance, indicated by a lowered responsiveness to the drug, coupled with a dependence characterised by a heightened responsiveness to opiate antagonists and disturbance on withdrawal of drug. A basic requirement for understanding the molecular mechanism of this remarkable phenomenon is to identify the cellular site at which it happens. As long as the phenomenon was studied only in whole animals, as it was with few exceptions until quite recently, there was little opportunity of identifying its site, although some experiments pointed towards it. The recent development of methods of studying opiate dependence and tolerance in the myenteric plexus of guinea pig ileum, in individual neurones of the nervous system *in situ* and in cultures of cells of neural origin has made it possible to identify the main cellular site at which opiate dependence and associated tolerance occur. This article presents and discusses implications of the evidence that this phenomenon occurs within the opiate-sensitive neurone. In this discussion the term 'opioid' will be used to include both opiates, which are analgesic derivatives of opium, such as morphine, and synthetic or endogenous substances having similar actions.

Opiate-sensitive neurone

The opiate-sensitive neurone is one that possesses specific opiate receptors, of any variety. It can be characterised in three ways. First, interaction between opiate molecules and these receptors produces a change in the neurone that results in the agonist actions of the drug. Second, this interaction between opiate and receptor can be blocked by the pure opiate antagonist, naloxone¹. Third, these actions are stereospecific, or perhaps more correctly, stereoselective, in that they are exerted by small doses of only the (–) enantiomers of opiates or opiate antagonists.

Such actions of opiates contrast with their nonspecific actions, which are not antagonised by, and may be shared with, naloxone. Thus, both morphine and naloxone, being phenols, can act as cofactors for prostaglandin synthetase². Again, both drugs inhibit hyperalgesia of rat paw elicited by intraplantar injection of prostaglandin E₂ (ref. 3). Non-stereospecific actions are produced by both the (+) and (–) enantiomers of opiates; for example, the same behavioural excitation is elicited by injecting either (+) or (–) morphine into the periaqueductal grey matter of rat brain⁴. This stimulatory action is not blocked by naloxone and is therefore also nonspecific.

Opiate-sensitive neurones exist in both the central nervous system (CNS) and the myenteric plexus. In the CNS, the action of opiates on the majority of these neurones is inhibitory; but a minority appears to be excited by opiates. For example, in a recent survey of the responses of individual neurones *in situ* in different areas of the rat brain⁵, the opiate drug, normorphine, and the opioid peptides, β -endorphin and Met-enkephalin, inhibited nerve impulse production in the majority of responsive

cells in the brainstem, cerebral cortex, caudate nucleus and thalamus; whereas each substance excited the hippocampal pyramidal cells. In the myenteric plexus, opiates inhibit the cholinergic neurone supplying the longitudinal muscle of the guinea pig ileum^{6,7} whereas they stimulate a neurone supplying the circular muscle of the rat colon^{8,9}.

Tolerance or dependence is usually observed towards the specific inhibitory actions of opioids; but it has also been described towards their specific stimulatory actions, both in the CNS^{10,11} and in the myenteric plexus^{8,9}. Since the above experiments on pyramidal hippocampal cells were reported, however, evidence has been obtained that their apparently specific stimulation by opioid is in reality a disinhibition of the adjoining basket cell, which itself maintains a tonic inhibition of the pyramidal cell¹². Possibly the other specific stimulatory actions of opioids also may eventually prove to be disinhibitions. Because of this, and because of the greater wealth of experimental data, only dependence and associated tolerance towards the inhibitory actions of opioids will be discussed below.

Before we enter this discussion, two principles should be noted. First, the response to binding of opiate molecules with specific receptors occurs at two rates—acute and subacute (or chronic). The acute response to a single dose may be assumed to begin immediately after the administered opiate has reached and bound with its receptor. The duration of the acute response can be measured in minutes. The subacute response to continued treatment, expressed as tolerance and dependence, is apparent within hours of treatment beginning. The duration of the subacute response has a briefer component, measurable in hours, and a longer component, measurable in days^{13,14}. Very prolonged effects of chronic treatment have also been observed, but they are not included in the present discussion. Second, in experiments on tolerance and dependence, a fundamental principle of uncertainty operates¹⁵. Tolerance cannot be measured directly without administering drug, nor dependence without withdrawing it. Hence we cannot measure tolerance and dependence in the same preparation at the same time. Measurement of tolerance and dependence separately in parallel preparations, however, has led to general agreement that opiate dependence is accompanied by tolerance, although the extent of the decrease in responsiveness to opiate (tolerance) may be numerically less than that of the increase in responsiveness to opiate antagonist (dependence)¹⁶.

Experiments in whole animals

Attempts to determine whether neurotransmitters were intimately involved in the mechanism of opiate dependence provided probably the earliest evidence on its cellular site, although their meaning was not clear to us at the time. In 1972, we showed that, in the dependent rat, naloxone elicited 10 behavioural signs of precipitated withdrawal, all of which were lessened or abolished by giving heroin shortly before naloxone

challenge¹⁷ (Table 1). In contrast to heroin, substances such as atropine, indomethacin and *para*-chlorophenylalanine, that block the action or formation of putative messengers between neurones, inhibited some signs of abstinence, intensified others and left still other signs unaffected. For example, atropine, given 30 min before withdrawal was precipitated by naloxone, significantly decreased the incidence of jumping, diarrhoea and chewing, but significantly increased that of irritability to touch and paw tremor, and left unchanged the incidence of teeth chattering, ptosis, head shakes and body shakes (Fig. 1). Essentially similar results were obtained when the withdrawal syndrome was modified with an inhibitor of the synthesis of serotonin or of prostaglandins. A simple and attractive explanation of these observations is that dependence in the whole animal occurs within neurones of the CNS that bear specific opiate receptors, and that other neurones with which these communicate are only secondarily affected.

Naloxone inhibits the induction of dependence, indicating that induction is specific, although a relatively high dose is needed¹⁸. That sensitivity to naloxone greatly increases in dependence^{16,17,19} also accords with dependence occurring within the opiate-sensitive neurone, since the amount of naloxone required to precipitate withdrawal in dependent states is so low that the naloxone is presumably acting only at the opiate receptors on the neurones.

Table 1 Blockade by heroin, given before challenge, of morphine withdrawal signs elicited by naloxone challenge in dependent rats¹⁷

Withdrawal sign	No. of rats positive Saline	No. of rats positive Heroin	Significance (<i>P</i> value)
Jumping	8	0	<0.001
Teeth chattering	10	1	<0.001
Irritable to touch	5	0	<0.02
Irritable to handling	10	1	<0.001
Diarrhoea	12	0	<0.001
Chewing	12	0	<0.001
Ptosis	11	4	<0.005
Body shakes	2	0	=0.24
Head shakes	5	1	=0.08
Paw tremor	3	0	=0.11

Twenty-four rats were made dependent by subcutaneous (s.c.) injection of 150 mg per kg of a sustained-release preparation of morphine; 23.5 h after this injection, 12 rats received heroin 40 mg per kg s.c. and 12 received saline; all rats were challenged 30 min later with naloxone 1.0 mg per kg s.c. Observers of the rat behaviour were unaware of what treatment each animal had received.

Myenteric plexus of guinea pig ileum

In the isolated ileum of the guinea pig, opiates inhibit contraction of the longitudinal muscle elicited by electrical stimulation of the post-ganglionic cholinergic neurone^{1,6,7}. Continued exposure to opiate of the isolated ileum at 4 °C, 22 °C or 37 °C produces tolerance to this effect^{7,20-23}, and dependence, usually expressed as a contracture of the longitudinal muscle on challenge with naloxone²¹⁻²⁶. As in the whole animal, tolerance and dependence thus induced in the isolated ileum are specific, in that induction is inhibited by naloxone^{22,26}. Effects in the ileum are also stereospecific, in that tolerance and dependence are induced by the (–) but not by the (+) enantiomer of a pair of stereoisomeric opiates and that withdrawal contracture is elicited by (–) but not by (+) naloxone²²⁻²⁶.

Three properties of dependence in the isolated ileum point towards its site. First, that the withdrawal contracture is blocked by hyoscine or atropine^{21,23,26} places dependence within the myenteric plexus of neurones rather than in the longitudinal muscle. Second, that the ganglionic blocking drug, hexamethonium does not prevent the induction or expression of dependence^{22,23,26} places dependence in the post-ganglionic region of the plexus, in which the opiate-sensitive neurones are present. Third, that responsiveness to naloxone increases both with duration of exposure to opiate and concentration of opiate

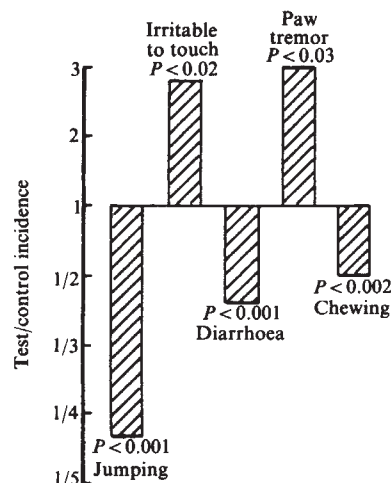


Fig. 1 Action of atropine on incidence of different withdrawal effects in morphine-dependent rats¹⁷. Withdrawal was precipitated by naloxone (1.0 mg per kg s.c.) 24 h after injection of a sustained-release suspension of morphine (150 mg per kg s.c.). Atropine (40 mg per kg s.c.) or 0.9% NaCl in water was given 30 min before naloxone. There were 32 test animals and 32 controls. The test/control incidence is the ratio of the incidence of the withdrawal effect in test and control groups. Atropine given before naloxone did not significantly change the incidence of teeth chattering, ptosis, body shakes or head shakes (Table 1).

used²⁶, until a very low concentration of naloxone is effective (Fig. 2), suggests, as already indicated, that the opiate-sensitive neurone is itself the site of dependence.

Individual neurones *in situ*

Opiate tolerance and dependence have been studied in individual neurones *in situ*, in the sensorimotor cortex of the rat brain²⁷ and in the myenteric plexus of the guinea pig ileum²³. In these studies, the action currents of nerve impulses were recorded by microelectrodes and drugs were applied by iontophoresis through micropipettes. Since neurones were used in which opiates inhibited the generation of nerve impulses and naloxone blocked the effect of opiates, these studies give direct information on tolerance and dependence in opiate-sensitive neurones.

In the sensorimotor cortex, continuous exposure to morphine for 40 min induced insensitivity to morphine, followed by a state of spontaneous excitation in the neurone that seems to represent dependence (Fig. 3). The inhibitory effect of morphine was also tested on cortical neurones stimulated with L-glutamate or acetylcholine in opiate-naïve and opiate-dependent rats. Whereas 63 of 83 cortical cells tested were inhibited by morphine in the opiate-naïve animals, only 1 in 50 was so inhibited in the dependent rats²⁷. Thus, there is a high probability that tolerance occurs in the neurones that are normally inhibited by morphine. Since these tolerant neurones became excited when treated with naloxone, we can infer that they were dependent also.

Table 2 Cyclic AMP production by NG 108-15 cells cultured for 48 h in the absence or presence of morphine (10 μM)

Challenge solution	Cyclic AMP produced by cells cultured Without morphine	Cyclic AMP produced by cells cultured With morphine
Control	21	23
Naloxone	21	37
PGE ₁	264	81
PGE ₁ + naloxone	241	1,183
Adenosine	103	65
Adenosine + naloxone	72	217

After 48 h incubation, cells were challenged by incubation for a further 10 min with addition of 10 μM naloxone, PGE₁ or adenosine, as indicated. Cyclic AMP was then extracted and estimated in pmol per mg protein. Data from ref. 29.

In the myenteric plexus of guinea pig ileum, post-ganglionic neurones were made tolerant and dependent by incubation with morphine, normorphine or levorphanol for 24 h at room temperature, in the presence of hexamethonium and hyoscine²³. In such neurones, administration of normorphine by micro-iontophoresis much less readily inhibited nerve impulse production than in control neurones (Fig. 4). In the absence of morphine, the neurone was spontaneously active, and, when treated with naloxone, the dependent neurones fired impulses at high frequency.

Neuroblastoma × glioma hybrid cells

There is no doubt that a certain line of neuroblastoma × glioma hybrid cells (NG 108-15 cells), cultured in the presence of an opiate, develop a form of tolerance and dependence, expressed as an increased capacity to produce cyclic AMP^{28,29}. Table 2 illustrates this form of dependence, in which challenge with naloxone in the presence of PGE₁ generated five times as much cyclic AMP in cells cultured for 48 h in the presence of morphine as in those cultured in its absence.

Although these cells possess opiate receptors³⁰, and the affinity of opiates for these receptors is correlated with their ability to inhibit adenylate cyclase³¹, the extent to which induction of tolerance and dependence in NG 108-15 cells resembles that in normal neurones is uncertain, for three reasons. First, although NG 108-15 cells put out long processes, they are not arranged in a stable intercommunicating system, as are neurones of the CNS or myenteric plexus. Each NG 108-15 cell must be regarded as a distinct unit, functionally resembling its neighbours. Second, the NG 108-15 cells are of malignant origin and, unlike normal neurones, they reproduce themselves. Thus, in 10% calf serum in their usual culture medium, the cells double their number in about three days³². These cells, however, can develop their characteristic tolerance and dependence even when their reproduction is inhibited (W. A. Klee, personal communication). The third difficulty is to translate a change in level of cyclic AMP in the NG 108-15 cell into terms of excitation or inhibition of normal opiate-sensitive neurones. The different effects of adenosine illustrate this difficulty. In NG 108-15 cells, adenosine stimulates adenylate cyclase (Table 2) and thus acts acutely in the opposite direction to morphine²⁹. In contrast, in individual opiate-sensitive neurones of the corpus striatum of rat brain *in situ*, both adenosine and morphine,

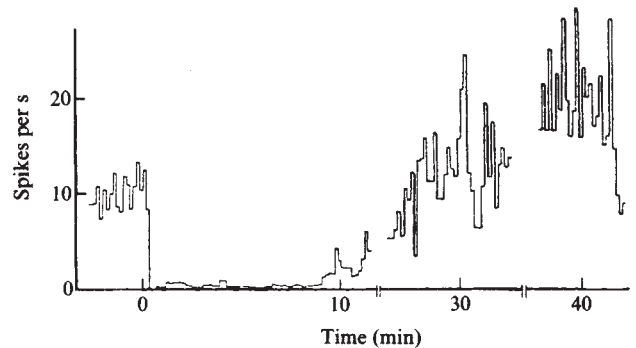


Fig. 3 Effect of prolonged application of morphine on frequency of nerve impulse production by a neurone *in situ* in the sensorimotor cortex of an opiate-naïve rat²⁷. Morphine was applied at 50 nA for 40 min from time zero. Breaks in the record are indicated by double vertical lines in the abscissa. For about 10 min, morphine suppressed nerve impulse production; thereafter production rose until, for the last part of the exposure to morphine, the neurone was producing impulses faster than it did before morphine.

administered by microiontophoresis, inhibit nerve impulse production³³. Moreover, in glial cells cultured from the brain of perinatal mice, adenosine binds with two distinct receptors³⁴. One of these inhibits adenylate cyclase and lowers the level of cyclic AMP, the other has the opposite effect.

It is tempting to suppose that, in the opiate-sensitive neurones of the rat striatum, the receptor activated by adenosine is one that inhibits adenylate cyclase. If so, the observed inhibition by adenosine of nerve impulse production in the striatal neurone might express a decrease in cyclic AMP; and hence a rise in cyclic AMP in the NG 108-15 cell might be translated as excitation in the opiate-sensitive neurone. This interpretation would be consistent with the specific and stereospecific inhibition of adenylate cyclase exerted by opiates and opioid peptides in homogenates or slices of brain *in vitro*³⁵⁻⁴², although their potency against adenylate cyclase and the potency of naloxone in antagonising their action are usually lower than might be expected from the affinities for opiate receptor in brain homogenates⁴³⁻⁴⁵. Attempts have so far failed to show, however, that opiates actually inhibit nerve impulse production in individual neurones of guinea pig myenteric plexus⁴⁶ or of cat spinal cord⁴⁷ *in situ* through an inhibition of adenylate cyclase.

Effects outside the neurone

The foregoing evidence shows that opiate dependence and associated tolerance can occur within the opiate-sensitive neurone; but it does not unequivocally indicate whether this neurone is their only site. That, as dependence deepens, preparations both *in vivo*^{16,17,19} and *in vitro*²⁶ become responsive to very low concentrations of naloxone suggests that the opiate-sensitive neurone is also the primary and essential site of dependence.

There is, however, evidence that changes in other neurones may reinforce the effect in opiate-sensitive neurones. Thus, continued treatment of rats with morphine induces a supersensitivity to stimulation of adenylate cyclase by catecholamines in a cortical neurone downstream of that inhibited by opiate⁴⁸. This supersensitivity is associated with an increase in post-synaptic receptors.

The opiate-sensitive neurone in a nervous system is necessarily subject to many influences from other neurones. Some of these could modify the induction or expression of dependence within the opiate-sensitive neurone. Such factors would include a diminished supply of endogenous opioid⁴⁹ or increased production of an endogenous opiate antagonist^{50,51}. Since dependence can be induced, however, in the isolated ileum, these factors may be restricted in origin to cells in the neighbourhood of the opiate-sensitive neurone, or to the neurone itself.

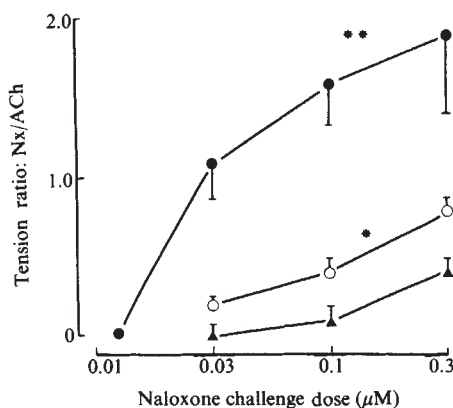


Fig. 2 Responses to naloxone challenge of segments of guinea pig ileum incubated for 24 h at $22 \pm 2^\circ\text{C}$ in Krebs solution containing $70 \mu\text{M}$ hexamethonium ($\blacktriangle$) or in this incubation medium, with the addition of $0.01 \mu\text{M}$ normorphine ($\circ$) or of $1.0 \mu\text{M}$ normorphine ($\bullet$) (ref. 26). Tissues were set up for test at 37°C in medium equivalent to that used for incubation and challenged 30 min later with naloxone (Nx). Tension ratio is the ratio of the maximal tension of contracture elicited by the challenge dose of Nx to that elicited by a reference dose of $0.01 \mu\text{M}$ acetylcholine (ACh). * $P < 0.01$, ** $P < 0.001$ for significance of difference of dose-response curve between normorphine-treated and control preparations.

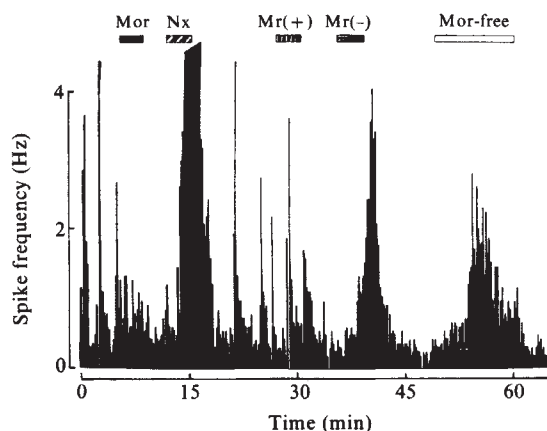


Fig. 4 Effect on a myenteric neurone of prolonged exposure to morphine in conditions of synaptic transmission blockade²³. Before beginning the recording (time zero), this neurone had been exposed for 24 h to a solution containing morphine (1 μ M), hyoscine (1 μ M) and hexamethonium (300 μ M). This solution continued to perfuse the tissue throughout the period of recording except for the time indicated by the open bar, when it contained only hyoscine and hexamethonium. During the periods indicated by the other bars, the perfusing solution contained additionally morphine (to a final concentration of 10 μ M, Mor), naloxone (100 nM, Nx), Mr2267 (100 nM, Mr(+)) or Mr2266 (100 nM, Mr(-)). Note that the neurone was tolerant in that a very high concentration of morphine no longer depressed its firing. Naloxone caused a pronounced increase in firing (this reached 8–10 Hz), Mr2267 had no clear effect on the firing rate, but its active, (-) enantiomer, Mr2266 caused a marked excitation. This neurone was also excited simply by removing the morphine from the perfusing solution (Mor-free). In this and other experiments, second and subsequent exposures to antagonists did not cause as marked an excitation as did the first application.

Molecular mechanism of dependence

In the dependent neurone, the balance between excitation and inhibition has moved in favour of excitation. This is expressed both in an increased responsiveness to excitatory^{52–54} and a decreased responsiveness to inhibitory^{55,56} messenger substances. This change in balance seems to express an increased state of intrinsic excitation, shown by the high rate at which the dependent neurone fires nerve impulses either spontaneously or when challenged with naloxone (Figs 3 and 4). In the presence of such excitation, the growth of tolerance to endogenous opioid⁴⁹, possibly through induction of an enkephalinase⁵⁷, would be expressed as dependence.

This change in the balance of excitation may correspond with 'neuronal kindling' (ref. 25) or with 'supersensitivity' and 'subsensitivity' (refs 58, 59); but such terms do not provide a molecular mechanism for dependence. The principle that dependence occurs within the opiate-sensitive neurone enables us to discard several hypotheses of this mechanism and to retain for further consideration those concerning three aspects of neuronal function; (1) receptors for humoral messengers, whether opioid or non-opioid; (2) release of transmitter from the neurone terminal; and (3) second messenger systems connecting receptor binding and transmitter release. Although hypotheses, based on changes in receptors^{4,25,59,60} or transmitter release^{61,62}, have been advanced, there is little supporting evidence. There is, however, strong evidence for a change in a second messenger system as the molecular mechanism of dependence. In theory, inhibition by opiate or other inhibitory substance of a process in a second messenger system could lead to tolerance and dependence, through a compensating hypertrophy in the system. This was first proposed for the cyclic AMP system³⁵, because opiates specifically and stereospecifically inhibited the E prostaglandin-stimulated adenylate cyclase of rat brain homogenate.

As we have seen, a compensating hypertrophy of cyclic AMP production in response to inhibition by opiate of adenylate cyclase has been clearly demonstrated in NG 108-15 cells²⁹. There is evidence of four types that such hypertrophy also occurs in neurones of dependent animals. First, brain cyclic AMP production is raised in opiate withdrawal^{63–68}. Second, injection of cyclic AMP into a cerebral ventricle of the dependent rat intensifies withdrawal jumping after naloxone challenge⁶⁹. Third, withdrawal is accompanied by an increase in protein kinase activity in brain homogenates^{68,70}. Lastly, a 'quasi-morphine withdrawal syndrome' indistinguishable in appearance from a true morphine withdrawal syndrome, can be elicited in opiate-naïve rats by treatment with a potent phosphodiesterase inhibitor, such as 3-isobutyl-1-methylxanthine (IBMX), together with a low dose of naloxone^{71,72}. The potency of phosphodiesterase inhibitors in eliciting this syndrome correlates with their potency as inhibitors of cyclic AMP phosphodiesterase in rat brain homogenate⁷³. On the present evidence, therefore, an hypertrophy of the cyclic AMP system within the opiate-sensitive neurone in response to inhibition of adenylate cyclase emerges as the most likely explanation of opiate dependence.

Difficulties

Four seeming difficulties emerge from the above analysis. First, the extent to which sensitivity to morphine declines in the induction of tolerance and dependence is usually less than that to which sensitivity to naloxone increases¹⁶. Second, although naloxone is a potent antagonist of the acute actions of opiates¹, it rather weakly inhibits the induction of opiate dependence¹⁸ or the inhibition by opiate of adenylate cyclase^{35–42}. Third, in rat brain preparations *in vitro*, the potency of morphine as a specific inhibitor of adenylate cyclase^{35–42} is usually less than might be expected from its affinity for the opiate receptor^{43–45}, although this does not apply to NG 108-15 cells³¹. Fourth, in individual neurones of the myenteric plexus or of the cat spinal cord, administration of neither cyclic AMP nor IBMX antagonised the acute inhibition by opiate of nerve impulse production^{46,47}, despite the evidence that opiates specifically inhibit adenylate cyclase in brain preparations *in vitro*^{35–42}.

These difficulties may disappear with continued experimentation. For example, although IBMX did not antagonise the acute inhibition by opiate of nerve impulse production in individual neurones of the myenteric plexus⁴⁶ or spinal cord⁴⁷, another methylxanthine, aminophylline, was recently found to do so in neurones of rat striatum³³. If, however, the difficulties persist and sharpen, a second postulate may be needed. One possibility would be that a special variety of opiate receptor subserves dependence induction. Another would be that binding of opiate molecule with its receptor activates two distinct processes: one, the inhibition of adenylate cyclase that leads to dependence; the other, so far unidentified, leading to the acute agonist action. As usual, new insights raise new problems and require new experiments.

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ARTICLES

Climate and sea level in isotope stage 5: an East Antarctic ice surge at ~95,000 BP?

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Six high-resolution records correlated with marine isotope stage 5 suggest that substage 5c was essentially interglacial, and was terminated by a catastrophic cooling. Over sixty ^{230}Th dates indicate that the sea level in substage 5c rose to at least -2 m. Amino acid ratios, archaeology, pollen and lithostratigraphy suggest that the sea later jumped to about +16 m. The combination of the cooling and the large jump points to an East Antarctic ice surge, at ~95 kyr BP.

BUDD AND MCINNES¹ suggest that "periodic surging of the large ice sheets may have been the rule rather than the exception". In Antarctica, Budd and McInnes envisage minor surges every 5 kyr or so, and major surges, such as those considered by Wilson², Hollin³ and Flohn⁴, every 100 kyr or so. I have pointed out that surging in Antarctica would have caused distinctive jumps of sea level: large, fast (a few hundred years or very much less?), brief (a few thousand years?), cool and, in the special case of Wilson's suggestion that surges caused the Pleistocene ice ages, late in each interglacial. I reviewed some lithostratigraphic and biostratigraphic evidence for such a jump late in the last interglacial, from below 0 m to about +16 m in stable areas. In 1972, impressed with the cooling events in Fig. 1b, c, d, and with the Mediterranean and Red Sea dates below, I estimated 95 kyr BP as the most likely date for this jump⁵. Later facing the

evidence that the last interglacial s.s. was represented by marine oxygen isotope substage 5e and dated from about 125 kyr BP (ref. 6), I thought that if the jump was real it must date from roughly 120 or 115 kyr BP (ref. 7). I review here new evidence that the sea rose almost certainly to -2 m and probably to 16 m at about 95 kyr BP. Biostratigraphic evidence that the rise was late in 'the last interglacial' is explained by evidence that not only substage 5e but also 5c was essentially interglacial in character.

Climate

Figure 1a is an equatorial record of marine oxygen isotope stage 5. The global record of stage 5, based on nearly 100 cores, forms our best framework now for thinking about the last interglacial,

s.l.⁸. However, in the marine isotope record, substage 5c usually appears more 'interstadial' than 'interglacial'; possible reasons are given later. Figure 1b, c, d show the Gulf of Mexico⁹, Orgnac (France)¹⁰ and Camp Century (Greenland)¹¹ rapid cooling events, whose interpretation as the result of an Antarctic ice surge has already been discussed⁵. Figure 1e, f, g come from high resolution pollen diagrams which appear to cover stage 5. Figure 1e is from the Tenagi-Philippon, Greece^{12,13}, where the oak forest of Wijmstra's zone S 'Drama', reflecting temperatures "a little lower than they are today", changed to cold steppe, reflecting "an average summer temperature at least 5–8 °C lower than today" (ref. 14) in one counting level, here estimated at <1,000 yr. Figure 1f is from Vandalia, Illinois¹⁵. If Gröger is correct in assigning his zone 1 to the 'Illinoian', that is, isotope stage 6, then the possible forest to prairie 'catastrophe' at the end of his zone 2c correlates very well with the coolings above. Figure 1g is from Woillard's¹⁶ profile from Grand Pile, France, in which she recognises three distinct interglacials in stage 5 time. Her St Germain I interglacial correlates with substage 5c,

but in this case its exact position on the horizontal axis was uncertain. The deforestation at 1,380 cm seemed much higher in the profile than the initial deterioration in the detailed diagram of the vegetation. In Fig. 1, I considered the most important climatic break was at about 1,460 cm (where the still high *Carpinus* pollen would match that in the massive brickearth at West Thurrock, UK below⁷). Now Woillard has reported¹⁷ that there was indeed an abrupt vegetational and climatic break at 1,455 cm.

Two important points emerge from Fig. 1. First, except in Fig. 1a, it seems that the substage 5c climate was essentially interglacial at the localities discussed. This shows clearly in the physical evidence from Orgnac and Camp Century, is implied by Gröger for his zone 2c and is argued specifically by Woillard¹⁸ for her St Germain I. Two North American speleothem records suggest that substage 5c was actually warmer than 5e (ref. 19). Proglacial meltwater lakes in Antarctica were particularly widespread in 5c time (ref. 20, p. 182, Fig. 4). I avoid defining 'interglacial', and do not think that substage 5c was as warm as 5e in every part of the globe, but in view of the evidence above it is not surprising that the sea in substage 5c rose slowly to within 2 m or so of its present level. Second, in all six of the high resolution records, it seems that substage 5c was terminated by a 'catastrophic' cooling. At Camp Century, where the time resolution is best, the cooling may have occurred in <100 yr.

Sea level

That the Gulf of Mexico cooling precedes a prominent ash deposit there, makes an Antarctic surge rather than a volcanic outbreak the most probable cause of these possible coolings^{5,21}. In any case, this particular cause is subject to the sea-level test. Traditionally (see, for example, refs 22–26) two sea levels—at 6–8 and 15–20 m—are assigned to the last interglacial in stable areas away from, for example, plate boundaries and the Pleistocene ice sheets. In the past 15 yr over sixty ²³⁰Th/²³⁴U dates have been obtained from geomorphological features, chiefly reefs and notches, at the 6–8 m level, notably in the Pacific and Indian oceans²⁷ and in detailed studies in Aldabra²⁸, Oahu²⁹ and the Bahamas³⁰. The mean of these dates is 125 kyr BP, so that the 6–8 m level seems to be from early in the last interglacial. The 15–20 m level is less pronounced geomorphologically, and has been neglected in dating studies: does it really belong to stage 5, or is it from an earlier interglacial? On the basis of its associations with Mousterian archaeology and cool molluscs, many early authors attributed it to the end of the last interglacial and the beginning of the last glaciation (ref. 31, pages 1256, 1271). In 1965 I suggested that such a high late level might be explained glacio-eustatically by an Antarctic ice surge³. I now consider the evidence for this hypothesis which leads to the sea-level scheme of Fig. 2a.

The classical area for the study of Pleistocene sea levels has been the western Mediterranean and Morocco. Work beginning in the nineteenth century was reviewed by Osborn and Reeds³², who called the correlation of a 20–18 m shoreline, a Mousterian archaeology and the early Würm glaciation "the most firmly established of any". In this area, Stearns and Thurber^{33,34} conducted a pioneering ²³⁰Th dating campaign on molluscs. Ten of their results fell between 140 and 115 kyr BP and 10 between 95 and 75 kyr BP. Of their dates for which elevations are given, the mean for the three shells in the older group is 6.5 m, and for the five in the younger is 11 m. Even if their young sample L934E from 33 m is omitted, the mean for the remaining four is still 5 m. Young sample L787D from 10 m comes from a conglomeratic apron reaching above 18 m. Obviously, these results favour early schemes and the present paper. However, there are two criticisms.

First, Kaufman *et al.*³⁵ concluded that the ²³⁰Th method on individual molluscs was giving correct ages no more than half the time. But, as the 140–115 kyr BP group fits very well the 125 kyr BP mean of so many coral dates, it seems likely that the 95–75 kyr BP group also corresponds to some real event, and concentrations of dates in this range continue to be obtained

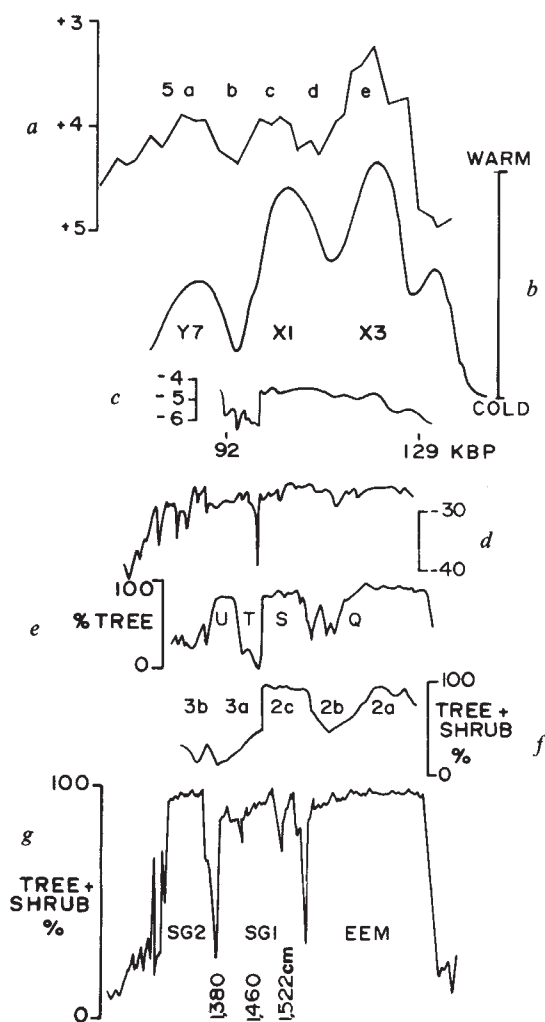


Fig. 1 Correlations with marine oxygen isotope stage 5. *a*, Isotope substages 5a–e in core V19-29 from east equatorial Pacific⁸. Scale on left is isotope composition of benthic foraminifera in ‰. *b*, Biological climatic curve from western Gulf of Mexico⁹. Y7 and so on are foram zones. *c*, Isotopic curve from Orgnac stalagmite, France¹⁰ in ‰. Numbers below are ²³⁰Th age determinations. *d*, Isotopic profile from Camp Century ice core, Greenland¹¹ in ‰. *e*, Pollen diagram from the Tenagi-Philippon, Greece^{12,13}. U and so on are pollen zones. *f*, Pollen diagram from Vandalia, Illinois¹⁵. 3b and so on are pollen zones. *g*, Pollen diagram from Grand Pile, France¹⁶. SG2 and so on are Woillard's St Germain II and I and Eemian interglacials. Numbers below are depths in cm.

from the Mediterranean³⁶. Especially important are the results of Bernat *et al.*³⁷ from Spain, where seven *Strombus* molluscs yielded a mean age of 97 kyr BP, with a standard deviation of only 4,900 yr. Sanlaville³⁸ describes a double transgression to 10 m in the Lebanon, associated with two mollusc dates of 90 kyr BP, and with archaeology from *le premier interstade würmien*. Second, the Mediterranean is a plate boundary area, and Hey³⁹, for example, has stressed the impermissibility of many of its raised shorelines. However, Gigout *et al.*^{40,41} claim that in the area of Bernat's dates, the lowest raised strandline can be traced for 400 of the 1,100 km they studied. We need more work in stable areas but, to test the surge hypothesis, it should be sufficient to see if the 95 kyr BP transgression was relatively higher than the 125 kyr BP.

Table 1 Summary list of ²³⁰Th/²³⁴U dates suggesting a substage 5c sea level at -2 m or above

Area	No.*	Dates (kyr BP)	Sea level (m)	Refs
Western Mediterranean,				
Morocco	10 M	95 to 75	18?	33, 34
Algeria	3 M	102, 93, 90		36
Spain	7 M	97 ± 4.9		37
Lebanon	2 M	90, 90	≥ 10	38
Black Sea	3 M	88 to 66	10 to 15	52
Egypt	4 C	92, 89, 89, 70	≥ 8	53
Madagascar	2 C	94, 85	≥ 1	54
Australia	8 M	101 ± 14		57
South-west Pacific	6 C	95 ± 4	≥ -2	61
Virginia area	11 S	84 to 59	14?	65
Florida	1 C	95 ± 9		68
Bahamas	4 C	112 to 94		30
Bermuda	1 C	97 ± 9	> 0	71
Canary Islands†	M	100	8?, 16?	86
Sudan	1 M	91	≥ 9, 17?	87
Mangaia	2 C	110, 90	> 2	27
Bonaire		103 (C) 93 (M)		88

* M, molluscs; C, corals; S, solitary corals.

† Last four areas are not discussed in the text, but merit study.

In the UK, I have summarised⁷ much evidence for a double sea level in the last, 'Ipswichian' interglacial: an early, 7-m level represented in the Thames estuary by brackish plants at Ilford, an inferred floodplain surface at Aveley, and tidally laminated beds at Crayford; and a late, 16-m level represented by a polleniferous aggradation at Aveley and West Thurrock, by Hodgson's sand and gravel sheet in Sussex, continuous from 0 to 14 m, and by the raised beach at Portland, rising to 20 m, and with modern but cool molluscs. Kidson⁴² suggests a double last interglacial sea level in Somerset, at 6–9.5 then 18–20 m, the later higher level represented by the Burtle Beds. Bowen⁴³ has long argued for a double level in Wales.

Recently, Miller *et al.*⁴⁴ reported an amino acid, relative dating campaign in the Thames estuary: modern D-alloisoleucine: L-isoleucine ratios (total, = free plus peptide-bound) are close to zero. Ratios from the freshwater mollusc *Corbicula fluminalis*, from Ilford, Aveley and Crayford above, fell into a well defined group with a mean of 0.21. Andrews *et al.*⁴⁵ reported on a similar study on coastal exposures. Ratios from the marine mollusc *Patella vulgata*, from Portland and the Burtle Beds, had a mean of about 0.12. The marine *Macoma balthica* from the Burtle Beds had a mean of 0.14. The small *Patella*-*Macoma* difference may be a species effect. But the large difference between *Corbicula* and these shells suggests that the latter may indeed be appreciably younger, especially as at the one locality where it is possible to compare the freshwater and marine ratios (Selsey-Selsea Bill; ref. 44, Tables 1, 2), four *Corbicula* all have ratios less than one *Macoma*.

An early 7-m level in the UK would correlate very well with the global 125 kyr BP, substage 5e level; is there any evidence

that a late 17-m level could be from a substage 5c interglacial? Sutcliffe has argued that the UK Ipswichian was at least twofold (see ref. 46, pages 223–224 for references and discussion). He claims that the mammals at Trafalgar Square, in particular *Hippopotamus amphibius*, show it to be from a younger interglacial than Ilford, Aveley and Crayford above. I have recently realised that *Corbicula* is missing from just those two Thames sites, Trafalgar Square and West Thurrock⁷, with *Hippopotamus*. In fact, a literature check has revealed no UK site where these two fluvial animals undoubtedly lived together. For example, there is no overlap between the seven sites in Sparks's⁴⁷ review of the last interglacial *Corbicula* and the four in Stuart's⁴⁶ review of last interglacial *Hippopotamus*. A third biological argument for multiplying the Ipswichian is provided by the contrast between the beetles from Aveley and the more exotic ones at Trafalgar Square (and Bobbitshole, Ipswich)⁴⁸. Woillard's¹⁶ diagram from Grand Pile suggests that it may be difficult to distinguish 5e and 5c interglacials by the pollen method, but Ilford and Aveley, Trafalgar Square and West Thurrock, plus the coastal sections should clearly be the subject of a further amino acid study.

A difficulty with this multiplication is that, if the *Corbicula* from Ilford, Aveley, Crayford (and Stutton^{7,44}) are from substage 5e, how can the massive brickearth at these localities⁷ be attributed to a surge in substage 5c? The recent pollen work of Woillard¹⁷, plus the sea-level observations of Chappell and Thom⁴⁹, raise the possibility of another (minor?) surge at the end of 5e also (Fig. 2a). (Rough calculations of the ensuing ice build-up rate in Antarctica (ref. 7, p 49) and perhaps the north suggest that a surge high sea stand probably lasted longer than Chappell and Thom assumed). If the resulting transgression rose to < 16 m, its littoral deposits would have been largely destroyed by the apparently major, 5c transgression that is the main concern of this article.

In northern France, Lower Normannian (up to 5 m) and Upper Normannian (12–18 m) beaches have traditionally been assigned to the last interglacial. Guilcher⁵⁰ writes "it may be that the Lower Normannian is older in some areas". In northern Europe, Charlesworth (ref. 31, p 959) reviews a large amount of evidence for a double sea level in the last interglacial. The second, 'Portlandia Sea', 'White Sea' transgression contains the *Carpinus* typical of the second halves of the Eemian and St Germain I interglacials. Van der Heide⁵¹ writes of the White Sea area "two transgressions are known. The first has a molluscan

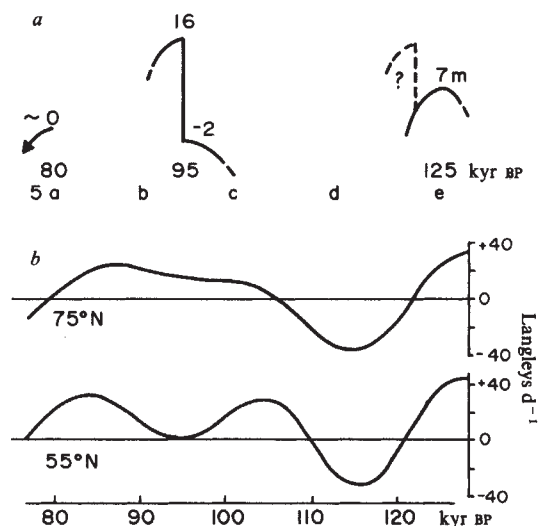


Fig. 2 a, Sea-level scheme for isotope stage 5, showing suggested major surge at about 95 kyr BP and possible minor surge at about 120 kyr BP. Figures above curves are elevations in m. 5a–e are correlations with isotopic substages. b, Deviations of solar radiation from their 1950 AD values, for the caloric Northern Hemisphere summer, for 75°N and 55°N, plotted from ref. 85, Table B.

fauna including somewhat warmer elements. The fauna of the second is markedly colder. Several authors believe that a connection between the White Sea and the Baltic Sea originated during this transgression". In the Black Sea, on the Caucasus coast, Muratov *et al.* (ref. 52, Table 1) assign to the Riss-Würm, Mikulino interglacial a double 'Karangat' transgression, to 5 then 10–15 m, associated with mollusc dates of 88–66 kyr BP.

In the Red Sea, Veeh and Giegengack⁵³ review several mollusc dates in the 90 kyr BP range, and report their own dates of 92, 89, 89 and 70 kyr BP from corals up to 8 m in Egypt. Battistini *et al.*⁵⁴ report coral dates of 94 kyr BP at 0 m and 85 kyr BP at 1 m from the area of Madagascar. From South Africa, near Port Elizabeth, Davies⁵⁵ reports that "Amino acid... ratios in molluscs... indicate that a transgression to +15 m occurred after a sea level at +8–9 m that is presumed to be Eemian in age on a variety of evidence. The transgression may be... from a surge of the... Antarctic ice sheet."

In Australia, in New South Wales, coral dates between 143 and 112 kyr BP are assigned to a sea stand at 5 ± 1 m (ref. 56). But in Victoria, Schornick⁵⁷ has reported eight mollusc dates with a mean of 101 ± 14 kyr BP. Outside New South Wales, the sea level in the last interglacial is thought to have been much higher; 21 m in Victoria⁵⁸ and 21 m by many workers in Bass Strait and Tasmania⁵⁹. As in Sussex above, the deposits involved seem to be unbroken down to below 0 m. That this level is slightly higher in Australia than elsewhere is what we might expect if the source of the water involved was (West or East) Antarctica (ref. 60, Fig. 4).

Table 2 Summary of data suggesting a stage 5 sea level at ~16 m

Area	Principal evidence	Refs
Western Mediterranean	Archaeology, ²³⁰ Th dates	32–34
UK	Lithostratigraphy, pollen, amino acid ratios	7, 42, 44, 45
Black Sea	²³⁰ Th dates	52
South Africa	Amino acid ratios	55
Australia	Lithostratigraphy	58, 59
Virginia	Lithostratigraphy, amino acid ratios	65, 67
Bermuda	Lithostratigraphy	70

In the Pacific, Bernat *et al.*⁶¹ report from New Caledonia and the Loyalty Islands four coral dates with a mean of 122 ± 3 kyr BP and six with a mean of 95 ± 4 kyr BP. Most of their samples were recrystallised, but the two with 100% aragonite came from the younger group. This area is close to a plate boundary, and the only island on which it is possible to compare elevations is Lifou. There, three of the older dates seem to come from within an old reef or cliff (the substage 5e one?), raised to about 11 m. One of the younger dates comes from the surface of the platform below this, at ~2 m. To test the surge hypothesis, it would be good to date more surface samples, below and especially above 11 m. In Japan, Kikuchi (ref. 62, Fig. 11) and Naruse have deduced double sea levels above 0 m in the last interglacial. Machida⁶³, working with fission track dates, estimated absolute sea levels at 100 and 80 kyr BP at about –9 to –3 m. Hopkins⁶⁴ deduced a double sea level in the last interglacial in Alaska, but thought that the early level was higher.

Marine deposits on the east coast of the US have been the subject of an extensive amino acid study by Belknap⁶⁵; his Fig. 44, on the Delmarva–Chesapeake Bay region, shows concentrations of dates at about 125 and 95 kyr BP (although the intervening unconformity at Wailes Bluff does not fit easily into Thompson's⁶⁶ pollen diagram). Many of these dates are from coastal exposures, and it seems likely that most of the shells from the thin, landward edges of the marine formations have been leached away. However, intensive lithostratigraphic work on the extent of these formations in Virginia was carried out by Oaks and Coch⁶⁷. They assigned to the last interglacial a Great Bridge Formation, related to a sea stand at about –2 m, which was

followed by an unusually rapid transgression to a Norfolk Formation level at 14 m. The sea then regressed below 0 m, leaving the deposits of the Kempsville, Londonbridge and Sand Bridge Formations. Five amino acid dates between 100 and 91 kyr BP seem to come from the Norfolk Formation. On the other hand, 11 solitary corals from the Norfolk and Kempsville Formations have a mean ²³⁰Th age of only 68 ± 9 kyr BP (ref. 65, appendix G). Whether this discrepancy has a geological or a chemical origin remains to be discovered. In Florida, coral dates include one of 95 ± 9 kyr BP from the Key Largo Limestone at Windley Key⁶⁸.

In the Bahamas (ref. 30, Table 2), perhaps the coral conglomerates dated to 103, 105, 94 and 112 kyr BP are reworked from a primary substage 5c sea stand. In Bermuda, Land *et al.*⁶⁹ recognised a sea-level maximum of 5 m at 120 kyr BP (four dates), then a regression to below 0 m, then a transgression to between 11 and 20 m, dated, however, to 130 kyr BP. Vacher⁷⁰ studied this second transgression more closely; he concluded that it was preceded by a sea level "slightly below present datum", that it reached 15 m and, from lithostratigraphic evidence, that it was "abrupt and rapid, and may signify a catastrophic surge in Antarctica". Harmon *et al.*⁷¹ confirmed the 120 kyr BP dating (mean of seven dates 124 ± 6 kyr BP), deduced a regression to –8 m or below then a second transgression at –6 m at about 100 kyr BP, redated the 130 kyr BP coral four times, and obtained a mean age of 97 ± 9 kyr BP for it. All these results fit Fig. 2a well. However, whereas the evidence for the 15-m sea level comes from seaward dipping calcarenites on the Bermuda north shore, the 97-kyr BP coral came from the south shore, from a deposit which Harmon *et al.* interpret merely as a storm beach, thrown up from a sea stand at about 0 m. Against any higher rise is the absence of any aragonitic, marine overgrowth on their speleothem 73018, from about 7 m and dating from 162 kyr BP. But perhaps this speleothem was protected against a brief transgression by the freshwater table. We need now some dates from the north shore calcarenites. However, in the surge hypothesis, these beds may represent, not conventional marine deposits, but rather the rapidly reworked, semi-consolidated terrestrial deposits and fossils from the 125–95 kyr BP regression: like the West Thurrock brickearth, a possible diluvium'.

Discussion

The ²³⁰Th dates above (Table 1) strongly suggest that sea level in substage 5c rose to at least –2 m. Many of the mollusc dates, plus the amino acid ratios, archaeology, pollen and lithostratigraphy (Table 2) suggest that the sea later rose to ~16 m. The data from Sussex, Portland and the White Sea suggest that the later rise was cool. Thames, Virginia and Bermuda authors have all argued that it was rapid. Unusually rapid rises to 15 m have been argued for Guernsey⁷² and Cyrenaica (ref. 73, p 54), although here no dating is available. Obviously, these data support the surge hypothesis. However, they also raise several questions.

(1) An immediate worry with Fig. 2 is the great size of the sea level jump: 18 m. The present distribution of the world's ice, in terms of sea-level equivalents, is roughly: East Antarctic ice sheet 60 m, West Antarctic ice sheet 6 m, Greenland ice sheet 6 m, lesser ice caps and valley glaciers 0.3 m. If the jump is real, then it requires a surge, or ungrounding (ref. 74, p 185), of the world's thickest ice, most probably from Wilkes Land, less probably from Queen Maud Land (see ref. 75 for bedrock and ice thickness maps).

(2) Veeh²⁷ puzzled why little trace of Stearns and Thurber's younger dates appeared in his coral study. We see now that the primary 5c reef, at ~–2 m, is out of sight today in stable areas. And the secondary, surge transgression would have been too rapid for reef growth, and would be marked chiefly by a sheet or patches of sand, with relatively cool molluscs. However, as the water cleared, and the initial cooling waned, some coral growth would have recommenced in low latitudes, and this is perhaps what we see in the Red Sea, Madagascar, and so on.

(3) Many workers remark that their elevations do not fit 'the Barbados model' (ref. 76), of 122, 103 and 82 kyr BP sea stands at +6, -13 and -13 m respectively. But Stearns⁷⁷ re-evaluated the Barbados (and New Guinea⁷⁸) elevation data, and concluded that the 103 and 82 kyr BP stands were in fact close to 0 m. The present data strongly support him. In a third uplifted area, in Atauro (ref. 79, Fig. 9), if reef terrace 1b in fact continued developing until 95 kyr BP it would reflect an absolute elevation not far below the -2 m given here.

(4) The Barbados model gained support from an early foraminiferal oxygen isotope study⁸⁰, which suggested a substage 5c stand at about -13 m. Subsequently, Barbados mollusc isotope data have suggested a 5c stand at -28 m, and Barbados coral isotope data a 5c stand at -43 m (ref. 81). Perhaps these disagreements can be resolved by interpreting the isotopic data more in terms of temperature than ice volume. The mean isotopic change, from substage 5e to 5c, involved in the results above was about +0.4‰. In the present scheme, the sea dropped from +7 to only -2 m in this time, so we can attribute only +0.1‰ of the change to an ice volume effect⁸. The remaining +0.3‰ may represent a small cooling, which we may calculate⁸² as 1.3 °C. Such a cooling in this area is indeed suggested by Kennett and Huddleston's planktonic biological data, in Fig. 1b.

(5) An even lower substage 5c stand is suggested by the 5e to 5c change of +0.7‰ in the benthic isotopes of Fig. 1a. This disagreement between stage 5 sea levels and the benthic isotope data has already been discussed, by Broecker⁸³. Again, he suggests a cooling, by about 2 °C, late in substage 5e. He draws attention to a possible analogous benthic change of over +0.2‰ late in the Holocene stage 1, a change which obviously cannot be interpreted in terms of ice volume.

(6) The emphasis here has been on finding out if Antarctic surges do in fact happen, and it may be premature to discuss their possible role in the spectrum of climatic change^{2,84}. However, one climatic point may be made. Not only a catastrophic cooling at the end of 5c, but also the whole of the subsequent cold substage 5b is hard to explain by insolation changes. During 5b time summer insolation north of 55° N was greater than today's, and north of 75° N was continually increasing (Fig. 2b). Substage 5b seems to be an ice age that is better explained by a surge than by insolation changes. Hitherto, cold substage 5d may have seemed a case of the opposite (Fig. 2), but now, in the light of refs 1, 17 and 49, and of the possibility of a minor surge at ~120 kyr BP, this no longer seems certain.

The sea-level scheme of Fig. 2 incorporates a very large quantity of data. We now need more quality. Above all, we need ²³⁰Th coral dates up towards 16 m in stable areas, although care must be taken to distinguish the possibly thin and patchy deposits of a surge from the older deposits and geomorphology beneath. It is just possible that the uppermost littoral deposits at 16 m are the results of the minor, substage 5e (120 kyr BP) surge suggested above, but most of the present evidence favours their attribution to a major, 5c, 95 kyr BP surge. If the surge hypothesis is wrong, its disproof could come deposits at about 10 m that remained continuously freshwater after 125 kyr BP (see refs 3 and 5, and Bermuda speleothem 73018 above).

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Enriched mantle: Nd and Sr isotopes in diopsides from kimberlite nodules

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Nd and Sr isotope compositions of diopsides from micaceous garnet lherzolite nodules found in kimberlite pipes at Bultfontein and Kimberley, South Africa are reported. The diopside data extend the mantle array, as defined by uncontaminated oceanic and continental basalts, to radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ values. These data are used to demonstrate the ancient enriched, grossly heterogeneous nature of the sub-continental mantle, which complements the predominantly depleted nature of the sub-oceanic mantle.

THE chemical character of the sub-continental mantle is the subject of considerable speculation. Most of our knowledge comes from heat flow and seismic data^{1,2} or analysis of mantle-derived materials. Thermal and seismic studies point to a continental mantle more depleted in magmatophile elements than the sub-oceanic mantle². Yet one of the principal conclusions from the interpretation of continental basalt data is that the continental lithosphere is essentially undepleted³ in the light rare earth elements (REE). However, note that interpretation of the Sr and Nd isotope geochemistry of continental volcanic rocks is severely hampered by uncertainties concerning contamination by crustal material^{4,5}. Thus considerable controversy has arisen over the extent of contamination and whether or not the range in isotopic composition reflects source heterogeneity³ or variable degrees of crustal assimilation^{4,5}. Recent interpretations of Rb–Sr isotope geochemistry assume that contamination effects are negligible³ but combined Nd- and Sr-isotope studies have elegantly demonstrated 'selective' contamination of basalts with crustal granulites and amphibolites. To alleviate these problems one can study peridotite nodules believed to be of mantle origin. However, care must be taken as minor amounts of contamination undoubtedly occurred during or after transportation of the mantle fragments to the surface. For example, recent isotopic studies of alpine peridotites have shown profound alteration during and after obduction⁶. Similarly, alkali basalt nodules are occasionally contaminated by the host basalt and some kimberlite nodules may have been 'metasomatised' by the host kimberlite^{7,8}. To eliminate any possibility of such contamination recent isotopic and trace elements studies^{6,9} have concentrated on carefully separated, inclusion free, mineral separates.

Diopside separates from these garnet lherzolites were chosen for analysis here because of their favourable abundances of Nd (20–50 p.p.m.) and Sr (150–400 p.p.m.), and the low Sm/Nd ratios (0.05–0.10), as well as the low Rb/Sr ratios (Table 1).

The analytical procedures used in this laboratory for Nd and Sr isotopes have been described elsewhere^{10,11}. Separation and purification procedures for the mineral fractions have also been outlined previously^{6,12}.

Nd and Sr isotopes in mantle diopsides

The Nd- and Sr-isotope compositions have been measured in diopsides for eight lherzolite nodules and the Nd isotopic composition in diopside from a ninth nodule (Table 1, Figs 1 and 2). The diopsides have a considerable range in $^{87}\text{Sr}/^{86}\text{Sr}$ = 0.70465–0.70752 and a similarly large range in $^{143}\text{Nd}/^{144}\text{Nd}$ = 0.51257–0.51195. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of co-existing garnet was measured in one nodule and was found to be identical to the co-existing diopside (Table 1). Secondary micas found in two

nodules have radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and low $^{143}\text{Nd}/^{144}\text{Nd}$ ratios (Table 1). The abundance of mica in these nodules can present a severe contamination problem due to the high Sr content (~500 p.p.m.). However, minute inclusions of mica in diopside would tend to produce a linear relationship between Sr content and $^{87}\text{Sr}/^{86}\text{Sr}$. This was not found to be the case and, furthermore, each diopside was carefully handpicked and inspected for adhering mica flakes or inclusions. The identical $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in co-existing garnet and diopside confirm the purity of our separates. It seems highly unlikely that random contamination of diopside (Sr = 140–400 p.p.m.) and garnet (Sr = 0.2 p.p.m.) with secondary mica flakes would result in identical $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. Contamination would tend to dramatically increase the Rb/Sr and $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the garnet and diopside.

The most interesting aspect of the Sr and Nd data for the diopsides is the linear anticorrelation of $^{143}\text{Nd}/^{144}\text{Nd}$ with $^{87}\text{Sr}/^{86}\text{Sr}$ (Fig. 1). The data extend the 'mantle array', that was originally observed in uncontaminated oceanic and continental basalts^{13,14}, to $^{87}\text{Sr}/^{86}\text{Sr}$ values of 0.7075. Clearly mantle-derived liquids can have high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios¹⁵ and low $^{143}\text{Nd}/^{144}\text{Nd}$ ratios. All of the diopsides represent mantle regions that have a time-integrated light REE enrichment (Sm/Nd ratio < the bulk earth value). Other available data on garnet lherzolite nodules^{13,16,17} reveal a Nd- and Sr-isotopic composition coincident with that of oceanic basalts. These lherzolites represent mantle with a time-integrated light REE depletion (Sm/Nd ratio > the bulk earth value). Clearly kimberlite nodules represent random samples of an inhomogeneous mantle characterised by both a LREE enrichment and depletion. Similarly data from alkali basalt nodules (ref. 18 and M.A.M. unpublished data) indicate that spinel lherzolite nodules represent mantle regions with both a time integrated light REE depletion and enrichment. These data have been summarised in Fig. 2 and compared with available basalt data.

Preliminary analyses of micas from garnet lherzolite nodules, plot off the mantle array as defined by the diopside data. Petrographic studies⁸ indicate a secondary origin for these micas, that may involve the host kimberlite. However, Nd isotopic studies¹⁷ of several fresh kimberlites indicate a source in the mantle with a chondritic REE abundance. This contrasts markedly with the two kimberlites analysed by DePaolo¹⁹. One highly serpentinised sample has a depleted source region while a slightly serpentinised sample is derived from an enriched source region. As these kimberlites are variably serpentinised their Nd and Sr isotopic compositions may reflect secondary processes. At this stage the mica data are difficult to interpret but we can say that a simple connection with the kimberlite magma is not indicated.

Sub-continental mantle

Ultramafic nodules found as spinel and garnet lherzolites in alkali basalts and kimberlites provide information on the isotopic and chemical constitution of the continental mantle. Mantle depletion and enrichment events will be recorded in these nodules throughout geological time. Rb–Sr^{9,20} and Sm–Nd^{16,21} measurements of minerals in some alkali basalt and kimberlite nodules record ages up to 3,000 Myr indicating that certain regions of the mantle have remained isolated for long periods of time. These ages may record ancient depletion events associated with continent formation, while Rb–Sr²² and Sm–Nd (M.A.M., unpublished data) studies of metasomatised nodules may record recent enrichment events (for example, ≈ 200 Myr) associated with alkaline magmatism. The consistent and wide variation in the Sr isotopic ratio of diopsides in mantle nodules is believed to reflect this gross mantle heterogeneity^{15,23}. The Sr and Nd data presented here support this contention and demonstrate that the sub-continental mantle contains regions with both time-integrated light REE enrichment and depletion (Figs 1 and 2).

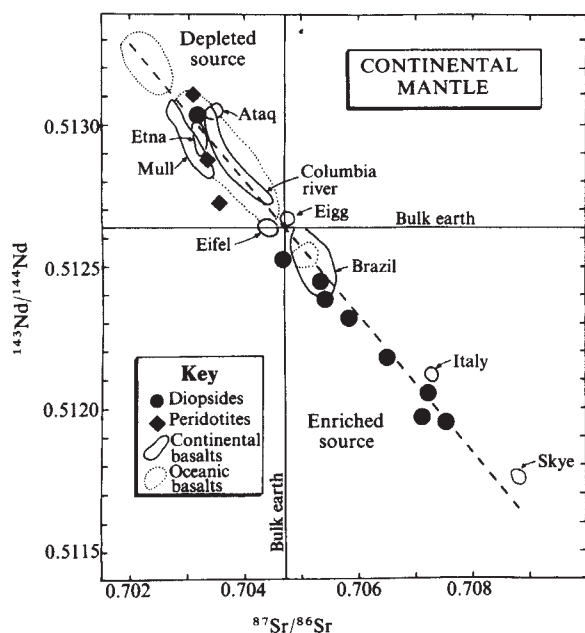


Fig. 1 $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ variation in diopsides from six kimberlite nodules (●) and one alkali basalt nodule from Nunivak, Alaska²⁴. Comparative sources: Ataq²⁴, Mt Etna and Roccamonfina²⁸; Mull, Eigg and Skye⁵; Eifel²⁷; Columbia River²⁵; and Brazilian flood basalts⁹. Whole rock kimberlite nodule data¹³ (◆) are shown for comparison.

Continental basalts are obviously derived from the sub-continental mantle and would be expected to reflect the gross heterogeneity described above. Figure 1 summarises available Nd and Sr isotopic data on continental basalts that are believed to represent uncontaminated mantle derived liquids. Basalts from Ataq, South Yemen²⁴; Mt Etna, Italy⁴; Columbia River, US²⁵; Rio Grande Rift²⁶ and other localities originated in depleted mantle regions similar to the source of oceanic basalts ($\text{Sm}/\text{Nd} > \text{the bulk earth value}$). However, continental flood basalts from Brazil¹⁹ and rift valley basalts from Eifel, Germany²⁷ are derived from enriched ($\text{Sm}/\text{Nd} < \text{the bulk earth value}$) and chondritic ($\text{Sm}/\text{Nd} \approx \text{the bulk earth value}$) source regions respectively. Nd and Sr data for uncontaminated basalts exhibit a considerable range in Nd and Sr isotopic composition that overlaps with that observed in diopsides from mantle nodules (Fig. 2). These data are therefore compatible with a

grossly heterogeneous sub-continental mantle containing both depleted residual and enriched portions.

To our knowledge, very few suites of 'uncontaminated' basalts have been reported with low $^{143}\text{Nd}/^{144}\text{Nd}$ and high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. In most cases contamination with a crustal component has produced marked deviations^{13,14} from the 'mantle array'. If we use the mantle array as a baseline against which contaminated suites can be compared, we may be able to define the approximate Nd and Sr isotopic composition of the 'mantle-derived liquid' that was subsequently contaminated during ascent through the continental crust. In the case of the Roccamonfina volcanic rocks from southern Italy²⁸ one sample (Fig. 1) has a Nd and Sr isotopic composition coincident with the mantle array as defined by the diopside data. Possibly this basalt represents a relatively uncontaminated volcanic rock derived from an enriched mantle. The associated volcanic rocks that plot to the right of the array represent mantle derived liquids variably contaminated with a crustal component²⁹, or uncontaminated liquids derived from metasomatised mantle²⁸. One can also speculate that the Nd and Sr isotopic composition of an epigranite from Skye, Scotland^{5,30} may approach that of the original mantle derived liquid. However, the chemically evolved nature of this rock indicates that it is a highly fractionated derivative of the original basalts. A closer look at the Skye volcanic rocks reveals two contaminated suites located in the upper left and lower right portions of Fig. 1. Carter *et al.*³⁰ suggest that the Skye volcanic rocks tapped a depleted mantle source but we suggest that perhaps the source was heterogeneous (that is, enriched and depleted).

Uncontaminated continental basalts, therefore, reflect dual mantle sources with both a time-integrated light REE depletion ($\text{Sm}/\text{Nd} > \text{the bulk earth value}$) and light REE enrichment ($\text{Sm}/\text{Nd} < \text{the bulk earth value}$). Similarly, careful investigation of contaminated suites of basalts in continental regions may provide evidence of enriched and depleted mantle regions. Basalt and nodule data are compatible with a grossly heterogeneous sub-continental mantle.

Table 1 $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ isotopic analyses of diopsides from micaceous garnet lherzolites found in kimberlite pipes

Locality	Rb/Sr	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{143}\text{Nd}/^{144}\text{Nd}^*$
Bultfontein B10 diopside	0.006	0.70646 ± 10	0.51216 ± 3
Bultfontein B10 garnet	0.315	0.70657 ± 8	—
Bultfontein B21 diopside	0.001	0.70537 ± 5	0.51237 ± 2
Bultfontein B69 diopside	0.001	0.70536 ± 9	0.51244 ± 3
Bultfontein B70 diopside	0.031	0.70702 ± 5	0.51195 ± 3
Bultfontein 017 diopside	—	—	0.51257 ± 2
Bultfontein 317 diopside	0.005	0.70722 ± 5	0.51206 ± 5
Kimberley CK26 diopside	0.015	0.70583 ± 5	0.51223 ± 2
Kimberley CK26 mica	3.723	$0.70569 \pm 6^\dagger$	0.51185 ± 7
Kimberley CK27 diopside	0.011	0.70465 ± 8	0.51250 ± 3
Kimberley CK27 mica	7.43	$0.72226 \pm 10^\dagger$	0.51224 ± 10
Kimberley CK32 diopside	0.003	0.70752 ± 7	0.51195 ± 4

* Normalised to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$.

† Corrected for radiogenic Sr growth for ~ 75 Myr, the approximate pipe emplacement age. $87_\lambda = 1.42 \times 10^{-11} \text{ yr}^{-1}$.

The compatibility of uncontaminated basalt data with mantle peridotite isotopic data indicates that perhaps these garnet and spinel lherzolites are typical of the source rocks of LIL enriched continental volcanic rocks. Trace element analyses of these peridotites, however, reveal an insufficient quota of LIL elements necessary for the production of basaltic and alkaline magmas^{6,7,31}. It is generally agreed that the source is an aluminous peridotite and, that the majority of garnet and spinel lherzolites have a trace element content that can, after addition of a LIL element enriched fluid, produce alkaline magmas. We have shown elsewhere¹² that $\approx 5\%$ metasomatism increases the trace element content of the source such that alkaline magmas

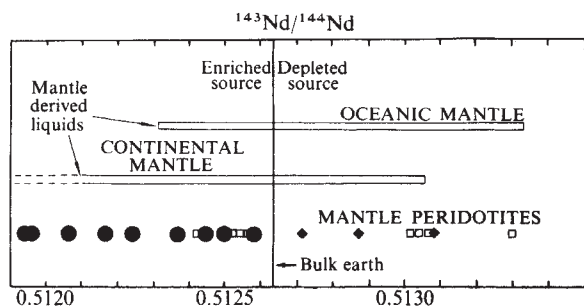


Fig. 2 $^{143}\text{Nd}/^{144}\text{Nd}$ variation in oceanic and continental basalts in relation to the mantle heterogeneity observed in diopsides from kimberlite (●) and alkali basalt nodules (refs 18, 24 and M.A.M., unpublished data) (□). Whole rock kimberlite nodule¹³ data (◆) are included for comparison.

can be extracted by 5–20% melting. Other authors^{32–34} have suggested that infiltration of metasomatic fluids provide the requisite amount of LIL elements and volatile fluxes that trigger anatexis. Therefore mantle peridotites containing accessory phases (amphibole, apatite, mica, sphene, and so on) are considered to be potential source materials. The diopsides analysed in this study are separated from multiply metasomatised garnet lherzolites containing both primary and secondary micas⁸. The metasomatised nature of the nodules is compatible with the isotopic data as metasomatism tends to lower the Sm/Nd ratio of the source region. Therefore phases with low Sm/Nd ratios would tend to record this enrichment event. (For comparison depletion events tend to increase the Sm/Nd ratio of the residual peridotite.)

In general there is clear evidence of recent or subrecent LIL and light REE enrichment of a metasomatic nature in the mantle source regions of oceanic and continental basalts (Fig. 1), but in the Nd and Sr isotopic data presented here we see the effects of ancient enrichment events in the sub-continental mantle. These metasomatic events are believed to be genetically related to upwelling of mantle plumes or blobs. Isotopic evidence for recent enrichment events is found in continental and ocean island basalts (Figs 1 and 2) that have $^{87}\text{Sr}/^{86}\text{Sr}$ lower than and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios greater than, the bulk earth value. This indicates derivation from source regions with a time-integrated light REE depletion (Sm/Nd greater than the bulk earth value). A depleted source contrasts markedly with the LIL enriched nature of the basalts and can best be explained by a recent enrichment event in the source region. This event presumably occurred so close to the time of eruption that it has not, as yet, been recorded in the $^{143}\text{Nd}/^{144}\text{Nd}$ ratio. Such recent events may have also affected the source regions of continental basalts from Nunivak island, Alaska, the Ataq diatreme, South Yemen¹², and the Eifel rift valley, Germany (Fig. 1). We have demonstrated elsewhere^{12,24} that in the case of Ataq recent enrichment or metasomatism is a precursor to continental alkaline magmatism. Rb–Sr data on basalts from the vicinity of Ataq may indicate that the event occurred 30–40 Myr before eruption of the basalts. Similarly Erlank and Shimizu²⁰ reported an age of 148 ± 7 Myr

for a metasomatic event in kimberlite nodules, that may represent a precursor to Karroo volcanism. Recent enrichment (231 ± 25 Myr) is also evident in metasomatically veined lherzolite nodules from Dish Hill, California (M.A.M., unpublished data). One can infer from this that enrichment events, or metasomatic precursors to alkaline (or tholeiitic) magmatism represent recent mantle events (≤ 250 Myr) superimposed on ancient, depleted, sub-continental mantle.

Enriched source regions resulting from such mantle enrichment (and associated diapirism) may temporarily be stored in the sub-continental mantle and adhere to the base of the continental (or oceanic) lithosphere³. Isotopic ageing will result producing the gross heterogeneities evident in the sub-continental lithosphere. However, note that these heterogeneities may result from original regional heterogeneities in the mesosphere³ if the plumes are derived from the mesosphere, or from mixing of mesospheric (lower mantle) and upper mantle components³⁷. Stored plumes may eventually be reactivated giving rise to basalts with radiogenically enriched $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios that reveal a time-integrated light REE enrichment in the source region (Sm/Nd < the bulk earth value). Such enriched sources, that are ancient in age, are found primarily below continents (Fig. 1) and are volumetrically insignificant in the oceanic mantle. For example, we find trivial amounts of undifferentiated mantle as plumes below only two oceanic islands (see refs 35, 36), whereas melting of ancient enriched mantle is more common in the source regions of continental flood basalts and other continental volcanic rocks (Fig. 1). Furthermore the relative predominance of enriched mantle in sub-continental regions complements the depleted nature of the oceanic mantle. Such a balance must exist somewhere if the assumption of a planetary distribution for the bulk earth is correct.

Conclusions

Hence, diopsides separated from kimberlite peridotite nodules exhibit a considerable range in Sr and Nd isotopes extending the mantle array to radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ values. It is evident from consideration of these data, and unpublished data on alkali basalt nodules, that the sub-continental mantle has been subjected to various enrichment events that produce a grossly heterogeneous mantle. The bulk of the sub-continental mantle may be depleted (as shown by the increase in Sm/Nd ratio) during continental formation, resulting in very old ages in most anhydrous residual nodules. However, the gross heterogeneity may result from the superposition of recent (≤ 200 Myr) and in some cases ancient enrichment events (for example, a decrease in Sm/Nd ratio) on the already depleted mantle. Regions of the mantle sampled by alkali basalts and kimberlites will therefore be characterised by both a time-integrated light REE depletion and light REE enrichment.

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γ - β -Thalassaemia studies showing that deletion of the γ - and δ -genes influences β -globin gene expression in man

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In γ - β -thalassaemia, human γ - and β -globin gene expression is suppressed; this results in a severe anaemia in newborns which subsequently develops into a β -thalassaemia syndrome in adult life. This hereditary disease is now shown to be the result of a deletion of at least 40,000 base pairs of the $\gamma\delta\beta$ -globin gene locus. The γ - and δ -globin genes are deleted in the affected chromosome but, surprisingly, the β -globin gene is still present, together with a large segment of the DNA sequences flanking the gene on its 5'-side and the entire region on the 3'-side of the gene. Hence, a deletion of DNA far from the β -globin gene results in the suppression of its activity.

THE human non- α -globin genes are clustered in a short region of chromosome 11. During fetal life, the major haemoglobin expressed is HbF ($\alpha_2\gamma_2$) where the γ -chains are coded for by two non-allelic γ -globin genes ($G\gamma$ and $A\gamma$). At about the time of birth, the expression of the γ -globin genes gradually ceases and HbF is replaced by HbA ($\alpha_2\beta_2$) and a low level of HbA₂ ($\alpha_2\delta_2$) (ref. 1). The fetal and adult genes are closely linked and gene mapping studies using Southern blotting² and the analysis of cloned genes have established the physical linkage of the δ - and β -genes^{3,4}, the two γ -genes⁵ and the tie up between these maps to describe the linkage between these four genes⁶⁻⁸. Figure 1 shows the physical map of the β -related globin genes.

Several well-defined defects in the functioning of these genes have been described. In addition to the abnormal haemoglobin proteins (such as HbS in sickle cell anaemia), inherited diseases collectively called the thalassaemias have been described in which the level of expression of these genes is altered. In the most common type, β -thalassaemia, two forms of the disease can be distinguished. In β^+ -thalassaemia, reduced amounts of β -globin are produced as a result of low levels of globin messenger RNA (mRNA)¹. In the second type, β^0 -thalassaemia, no β -globin is produced. β^0 -Thalassaemia is a heterogeneous disease and except for two forms of this disease⁹⁻¹¹ the molecular basis is unknown. The rarer disease, $\delta\beta^0$ -thalassaemia, is the result of a gene deletion (see ref. 1 for references) which inactivates both the δ - and β -globin genes. The deletion maps from the intervening sequence of the δ -globin gene^{6,12,13} to a site 1,800 base pairs past the β -globin gene¹³; altogether, 10,000 base pairs have been deleted¹³. Finally, in the condition HPFH (hereditary persistence of fetal haemoglobin) the entire $\delta\beta$ -globin gene region has been deleted (see ref. 1 for older references), spanning from sites 4 kilobase pairs in front of the δ -globin gene in two cases^{6,7} and 7 kilobase pairs in front of the δ -globin gene in another (R. Bernards and R.A.F., unpublished) to a position well past the β -globin gene.

Several other rare forms of thalassaemia affecting the β -related globin genes are known. In γ - β -thalassaemia, first

described by Kan *et al.*¹⁴, a severe anaemia is evident in newborns as a result of a reduction of the γ/α synthetic ratio to about 0.5. As γ -chain synthesis is switched off in the course of normal development, the disease develops into a mild β -thalassaemia which is unusual in that the HbA₂ ($\alpha_2\delta_2$) levels are normal, rather than elevated as in classical β -thalassaemia. To explain this, Kan *et al.*¹⁴ postulated that the entire $\gamma\delta\beta$ -region has been deleted in γ - β -thalassaemia.

We show here that the $G\gamma$ -, $A\gamma$ - and δ -globin genes have been deleted in γ - β -thalassaemia. Surprisingly, however, the β -globin gene is present, together with 2,500 base pairs on its immediate 5'-side and the entire 3'-extragenic region. Figure 1 compares the structure of the affected region in γ - β -thalassaemia with the corresponding region of normal DNA.

γ - β -Thalassaemia in a Dutch family

One of us (M.O.) has been involved in the treatment of a Dutch family which exhibits the same clinical symptoms as those originally described for γ - β -thalassaemia¹⁴. These data (including an extensive family study) will be presented in detail else-

Table 1 Haematological data and β/α specific activity ratios for γ - β -thalassaemia

	Patient 2	Normal females
Hb (g dl ⁻¹)	9.7	12.0-15.0
Erythrocytes ($\times 10^{12} l^{-1}$)	4.5	3.5-5.5
Mean cell volume of erythrocytes (fl)	67	82-94
Reticulocyte count (%)	5.2	0.5-1
HbA ₂ (% of total Hb)	3.1	1.9-3.0
HbF (% of total Hb)	1.0	0.1-1.5
β/α synthetic chain ratio	0.49	0.9-1.0

Haemoglobin was determined by the cyanomethaemoglobin method, the erythrocytes counted in a Hycell counter and the mean cell volume of erythrocytes calculated from the erythrocyte count and the haematocrit. The reticulocyte count was determined by microscopy and the HbA₂ and HbF determined as described in refs 20 and 21. The β/α synthetic ratios were determined by the incorporation of ³H-leucine by reticulocytes followed by the separation of the globin chains according to ref. 22. The specific activity of each chain was determined by hydrolysing the α - and β -globins and determining the leucine content directly. The values for normal females are the average values found in the laboratories of D.R. and L.B.

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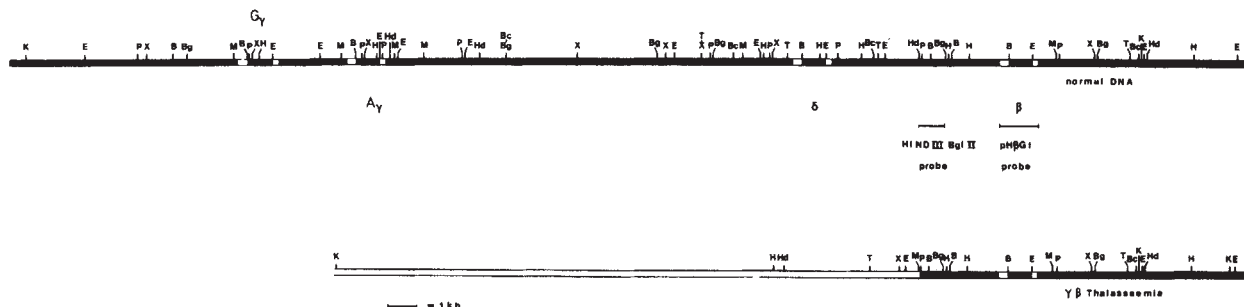


Fig. 1 Restriction map of the γ - β -thalassaemic globin genes compared with normal DNA. The probable positions of the coding regions are shown as open boxes. It should be stressed that only extragenic sites which can be detected for a given enzyme by the blotting analysis are those closest to the sequence complementary to the probe used. Certain sites between the δ - and β -globin genes are as originally described in ref. 6. The positions of the remaining sites are described in refs 8 and 13, and most were originally described in refs 3 and 5. The *MspI* sites are from L.H.T.V.P. and R.A.F. (unpublished). The *KpnI* site to the 5'-side of the γ -globin gene has been remapped at 7.5 kilobases upstream from the gene in *HpaI* + *KpnI* double digests. This means that the *KpnI* fragment is 39 and not 46 kilobases, as estimated from its electrophoretic mobility in agarose gels⁸. B, *Bam*HI; Bc, *Bcl*I; Bg, *Bgl*II; E, *Eco*RI; H, *Hpa*I; Hd, *Hind*III; K, *Kpn*I; M, *Msp*I; P, *Pst*I; T, *Taq*YI; X, *Xba*I.

where. Briefly, of a family with 13 progeny, 7 (now adults) were found to exhibit a β -thalassaemia minor with normal HbA₂ levels rather than the elevated HbA₂ characteristic of classical β -thalassaemia. Two of these adults have been analysed in the present article (patients 2 and 3). Some of the relevant haematological data for patient 2 are compared with the average data for normal individuals in Table 1. In particular, note that the β/α chain synthetic ratio was 0.49 in this case, rather than the 0.9–1.1 values found for normal individuals.

In the past 14 yr, nine infants, progeny of the seven affected individuals described above, have been examined by M.O. They showed the clinical course described by Kan *et al.*¹⁴ with severe postnatal haemolytic anaemia, a mixed hyperbilirubinaemia with both direct and indirect bilirubin being elevated; a drastic decrease in haemoglobin level occurred in the immediate post-natal period.

During postnatal development, a chronic microcytic anaemia persisted in all cases, with normal or near normal reticulocyte counts and bilirubin levels. The globin synthetic ratios have been measured in two affected infants, namely, a 4-month-old child of patient 2 and a newborn child of patient 3. In the first case the child showed 23% HbF, with a β/α ratio of 0.56 and a γ/α ratio of 0.74. In the second case 87% HbF was found, with a γ/α synthetic ratio of 0.5. Thus, γ - and β -gene expression is impaired in the same way as originally described by Kan *et al.*¹⁴. As the γ -lesion has only been observed in several children of the sibling adults with the β -thalassaemia trait (whose spouses were in all cases haematologically normal), there can be no doubt that the γ - β -thalassaemia lesion is carried on the same chromosome. (Normal infants have also been born in these families with neither γ - nor β -globin gene expression affected.)

Abnormal β -globin gene fragments in γ - β -thalassaemia

To analyse the globin gene structure in this disease, we isolated DNA from peripheral blood from two affected adult γ - β -thalassaemia heterozygotes (patients 2 and 3) and their normal spouses (1 and 4), digested the DNA with various restriction endonucleases and analysed the globin gene fragments by Southern blotting², followed by filter hybridisation^{2,15,16} as described previously^{3,4} using cloned human β and γ -complementary DNA (cDNA)¹⁷ as probes for the β - δ - and γ -globin genes, respectively (see Fig. 2 legend for details).

Hybridisation of *Bgl*II, *Xba*I and *Bam*HI-digested DNA from the γ - β -thalassaemia patients with γ -gene probes (pH γ G1)¹⁷ showed only the normal γ -globin bands described previously⁵ (Fig. 2). This must mean either that the γ -globin gene region is normal in γ - β -thalassaemia or that the entire γ -region has been deleted in the affected chromosome. The reduced intensity of the bands in the patients compared with the

controls suggested the latter. Using probes for the β -globin structural gene sequences (pH β G1 DNA)¹⁷, abnormal bands were seen in *Hind*III, *Eco*RI, *Xba*I, *Msp*I and *Taq*YI-digested DNA in addition to the normal bands derived from the unaffected chromosome (Fig. 2; Table 2). In contrast, the *Pst*I, *Bgl*II (Fig. 2) and *Hpa*I (Fig. 3) patterns for the δ - and β -globin genes of the thalassaemics were the same as for control individuals (Fig. 2; Table 2 gives the relevant sizes of the fragments). The *Bam*HI patterns for the control individuals and one of the patients are also the same; they do, however, differ from all other human DNAs that we have examined in that they contain a 22-kilobase 3' *Bam*HI β -globin gene fragment instead of the usual 8.6-kilobase fragment. The second patient is heterozygous for this *Bam*HI polymorphism; this DNA contains both the 22-kilobase and 8.6-kilobase 3' β *Bam*HI fragments. This *Bam*HI polymorphism is, however, not directly relevant to the γ - β -thalassaemia lesion because it is also found in individuals unaffected by the disease.

Inspection of the restriction map of the β -related globin gene locus (Fig. 1) suggests that a break has occurred in γ - β -thalassaemia in the 200-base pair region between *Hind*III and *Pst*I on the 5'-side of the β -globin gene: all cleavage sites on the 3'-side of this site are normal, whereas the *Hind*III, *Eco*RI, *Bcl*I, *Taq*YI, *Xba*I and *Msp*I sites on the 5'-side differ from the normal ones.

To confirm this, we hybridised the same DNA samples with the 0.9-kilobase *Hind*III + *Bgl*II fragment containing the DNA region 1.9–2.8 kilobases to the 5'-side of the β -globin gene, that is, the region of the putative break point. Figure 1 shows where this probe is located on the map. The *Bgl*II–*Hind*III probe detects all the abnormal ' β '-globin bands detected above and the corresponding normal β -globin fragments (Fig. 4). We have also mapped the 'abnormal' cleavage sites of those enzymes (*Bam*HI, *Hpa*I and *Pst*I) that gave apparently normal patterns with pH β G1 probes, because the cleavage sites mapped with pH β G1 are located between the β -globin gene and the break point in γ - β -thalassaemia. Thus, *Bam*HI gives a 0.7-kilobase band with both normal and γ - β -thalassaemia patient DNAs (Fig. 4); this 0.7-kilobase fragment is adjacent to the 1.8-kilobase *Bam*HI β -fragment at its 5'-terminus⁶. The break point must, therefore, be to the 5'-side of this (or alternatively, it is within this fragment but the new *Bam*HI site is coincidentally at the same relative position as the 'old' site). In DNA cut with *Hpa*I, a novel fragment of 5.6 kilobases is seen (in contrast to Fig. 3). DNA cut with *Pst*I still does not show any abnormal bands; only the 4.4-kilobase β -globin gene fragment is visible. We presume that we do not detect an abnormal *Pst*I fragment because the break point is essentially at the normal *Pst*I site; in this case, little or no homology would be seen between the fragment spanning the break point and our *Bgl*II–*Hind*III probe.

Mapping the size of the deletion in γ - β -thalassaemia

To confirm that the break point we have deduced above is indeed the correct explanation for the γ - β -thalassaemia fragment pattern, we have mapped several cleavage sites around the β -globin gene in double digests. The abnormal 5' *Eco*RI and *Msp*I sites have been mapped in *Pst*I + *Eco*RI and *Msp*I + *Eco*RI double digests; Table 2 shows that the data from these digests fit with the position of the break point already deduced.

All the data support a model in which a break in the chromosome has occurred between *Pst*I and *Hind*III 2.5 kilobases upstream from the β -globin gene. To determine whether a deletion or inversion has occurred, we analysed *Kpn*I digests. In normal DNA, *Kpn*I yields a single 39-kilobase globin gene

fragment containing the γ -, δ - and β -globin genes (Fig. 1, ref. 8). In DNA from the γ - β -thalassaemia patients, a second 27-kilobase band is visible which hybridises with β -globin probes but not γ -globin probes (Fig. 5). Apparently, a large deletion has occurred in γ - β -thalassaemia, eliminating the γ -globin genes entirely. The 3' *Kpn*I site was mapped in γ - β -thalassaemia DNA in *Kpn*I + *Hpa*I and *Kpn*I + *Bam*HI double digests. To our surprise, two 3' *Kpn*I sites are indicated in this experiment in both the patients and their normal spouses, who are also from this region of The Netherlands. The closest *Kpn*I site to the β -globin gene is 5.5 kilobases to the 3'-side of the 5' β -globin *Hpa*I site and corresponds to the 3' β -globin *Kpn*I site already mapped by us in several DNAs from normal Dutch, Indian and English individuals, an Italian Hb_{Lepore} patient and an Italian $\delta\beta^0$ -thalassaemia patient. The second site lies to the 3'-side of the *Hpa*I site downstream from the β -globin gene and,

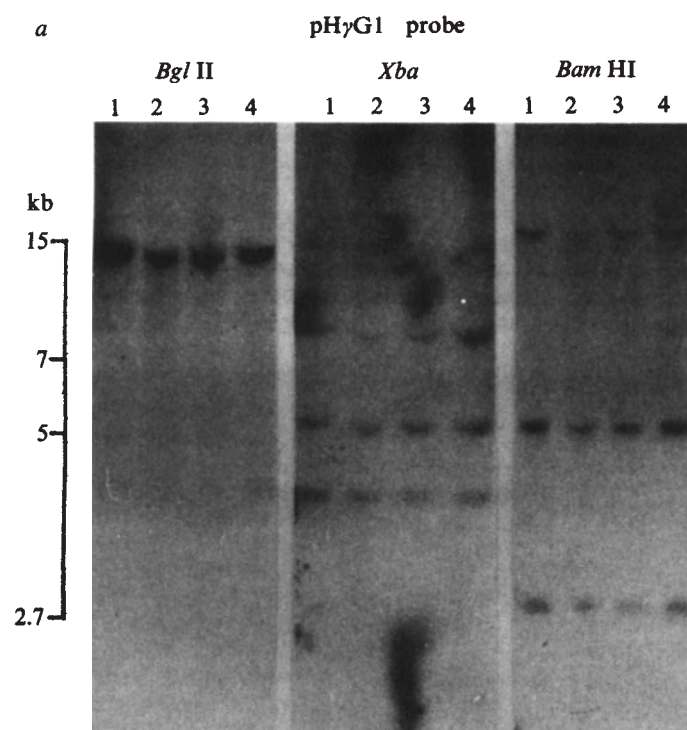
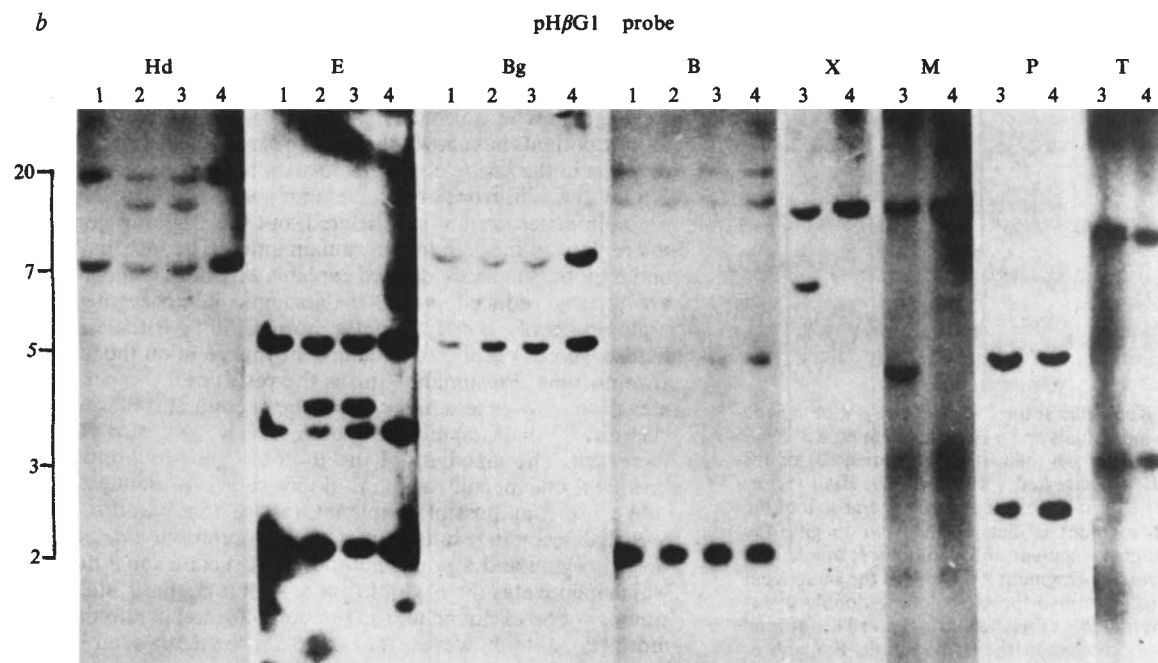


Fig. 2 γ -, δ - and β -globin gene fragments in γ - β -thalassaemia. DNA was isolated from peripheral blood of two γ - β -thalassaemic patients and their normal spouses as described³ except that a 30-min incubation in proteinase K (100 μ g ml⁻¹) in 1% SDS, 0.1 M EDTA and 0.15 M NaCl (pH 8.0) at 37 °C preceded the phenol extractions. DNA was digested with the relevant restriction endonucleases³ and about 10 μ g per lane was electrophoresed in 0.7% agarose gels for 36–48 h at 0.7 V cm⁻¹. The DNA was blotted² on to nitrocellulose filters and hybridised as previously described^{3,16} except that 9% dextran sulphate (Sigma, 500,000 molecular weight) was added to the pre-hybridisation wash and to the hybridisation solution²³. Hybridisation probes were pH β G1 (*a*) and pH γ G1 (*b*) for the $\delta\beta$ - and γ -genes, respectively^{3,5,17}. After a 12-h hybridisation (using 1 ng of ³²P-labelled DNA probe per ml; 4 $\times$ 10⁸ c.p.m. per μ g) at 65 °C, the filters were washed as described elsewhere³. Bands were detected by autoradiography for 12–48 h as previously described³. For abbreviations, see Fig. 1 legend. The two additional faint bands seen in the *Bam*HI–pH γ G1 hybridisation are partial digestion products.



therefore, only half the 7.7-kilobase *HpaI* band is cut (Fig. 3). The second polymorphic *KpnI* site is precisely located in the *BamHI* + *KpnI* double digest; a new 7.7-kilobase *BamHI* + *KpnI* double digest fragment places this site at 7.7 kilobases from the intragenic β -globin *BamHI* site, 3.1 kilobases past the 'normal' *KpnI* site (Fig. 3). Patient 3 and control 4 are, therefore, heterozygous for a 3' *KpnI* polymorphism as well as being homozygous for the 3' *BamHI* polymorphism described above.

Figure 1 compares the maps of the normal globin gene region with that of γ - β -thalassaemics. How large is the deletion in γ - β -thalassaemia? The minimal size of the deletion seems superficially to be 12 kilobases, that is, the difference in size between the normal *KpnI* fragment (39 kilobases) and the abnormal fragment (27 kilobases). Inspection of the map in Fig. 1 shows, however, that this cannot be the case. This would place the 5'-break point in the γ -globin gene region and would result in abnormal *BamHI*, *XbaI* and *BglII* γ -globin gene fragments; these are not seen. The explanation must, therefore, be that the deletion extends past the *KpnI* site on the 5'-side of the γ -globin genes; the entire γ -globin gene region is deleted in γ - β -thalassaemia. *HpaI* digests of DNA from γ - β -thalassaemics define the minimal size of the deletion. Because the γ -globin genes are completely deleted in γ - β -thalassaemia, the first *HpaI* site present on the 5'-side of the break point of the deletion must be past both γ -globin genes. Fritsch *et al.*⁶ have shown that the first

Table 2 Size of globin gene fragments in restriction enzyme digests

Enzyme	Fragment size		
	γ - β -Thalassaemia gene	δ -Gene	β -Gene
Detected with pH β G1 probe			
<i>HindIII</i>	12.0	17.0	7.6
<i>EcoRI</i>	4.2	2.3 + 1.9	5.8 + 3.7
<i>BglII</i>	NAB	8.4	5.0
<i>BamHI</i>	NAB	15.0 + 4.3	1.8 + 22 or 8.6
<i>XbaI</i>	6.4		10.8
<i>MspI</i>	4.5		10.5
<i>PstI</i>	NAB	2.3	4.4
<i>TaqYI</i>	9.0	8.6	3.1
<i>PstI</i> + <i>EcoRI</i>	NAB	1.7 + 0.6	3.6 + 0.8
<i>HpaI</i>	NAB	1.9 + 1.4	7.8
<i>EcoRI</i> + <i>MspI</i>	3.7	2.3 + 1.9	5.8 + 0.8
<i>KpnI</i>	27		39
<i>KpnI</i> + <i>HpaI</i>	NAB	1.9 + 1.4	7.8 or 5.5
<i>BamHI</i> + <i>KpnI</i>	NAB	15.0 + 4.3	7.7 or 4.6
Detected with <i>HindIII</i> - <i>BglII</i> probe			
<i>HindIII</i>	12.0	—	7.6
<i>EcoRI</i>	4.2	—	5.8
<i>BamHI</i>	NAB	4.3	0.7
<i>XbaI</i>	6.4		10.8
<i>HpaI</i>	5.8	—	3.1
<i>KpnI</i> + <i>HpaI</i>	5.8	—	3.1

The γ - β -thalassaemia fragments are from the present paper. The β - and δ -globin gene fragments have been reported previously^{3,6,8,13}. NAB, no additional bands.

HpaI site to the 5'-side of the γ -globin gene is 23 kilobases upstream from the gene in normal DNA and we have confirmed this in several normal individuals. Let us assume that this is the 5' *HpaI* site of the abnormal 5.6-kilobase *HpaI* fragment in γ - β -thalassaemia. At its 3'-end the 5.6-kilobase fragment must contain the 0.9 kilobases of DNA which extend from the 3'-break point of the deletion to the *HpaI* site in front of the β -globin gene. The closest position for the 5'-break point of the deletion is, therefore, 4.7 kilobases (5.6-0.9) from the '5' γ ' *HpaI* site; inspection of Fig. 1 shows that this predicts a minimal deletion of 42 kilobases. Maniatis and his colleagues have recently shown that an embryonic globin gene is located to the 5'-side of the γ -globin gene; as this ϵ -gene is 8 kilobases upstream from the γ -globin gene (T. Maniatis, personal communication) we conclude that this gene is deleted in γ - β -thalassaemia.

Discussion

Comparison of the detailed maps of restriction endonuclease cleavage sites in normal DNA³⁻⁸ and DNA from γ - β -thalassaemic patients has shown that this disease is the result of a large deletion in the $\gamma\delta\beta$ -globin gene locus which has its 3'-end point 2.5 kilobases in front of the β -globin gene. The γ - and δ -globin genes are entirely deleted, but the β -globin gene and the regions to the 3'-side of β remain intact. The fact that the γ - and δ -globin genes are deleted explains why both gene products are grossly reduced in γ - β -thalassaemic heterozygotes. The δ -globin levels are apparently normal in γ - β -thalassaemia heterozygotes despite the deletion of this gene on the affected chromosome. Presumably, this is the result of a compensatory elevation of δ -gene activity from the second chromosome; in classical β -thalassaemia, δ -globin levels are also usually increased. The inactivity of the β -globin gene in adult life is, however, unexpected, as the β -globin gene is present.

We see four possible explanations for this paradox. First, γ - β -thalassaemia results from a double mutation, a deletion of the $\gamma\delta$ -region and a point mutation or a second small deletion which inactivates the β -globin gene. Although this is unlikely it cannot yet be excluded and so remains a formal possibility. It is more probable, however, that the β -thalassaemia seen in γ - β -thalassaemics is the result of the deletion mapped in this article.

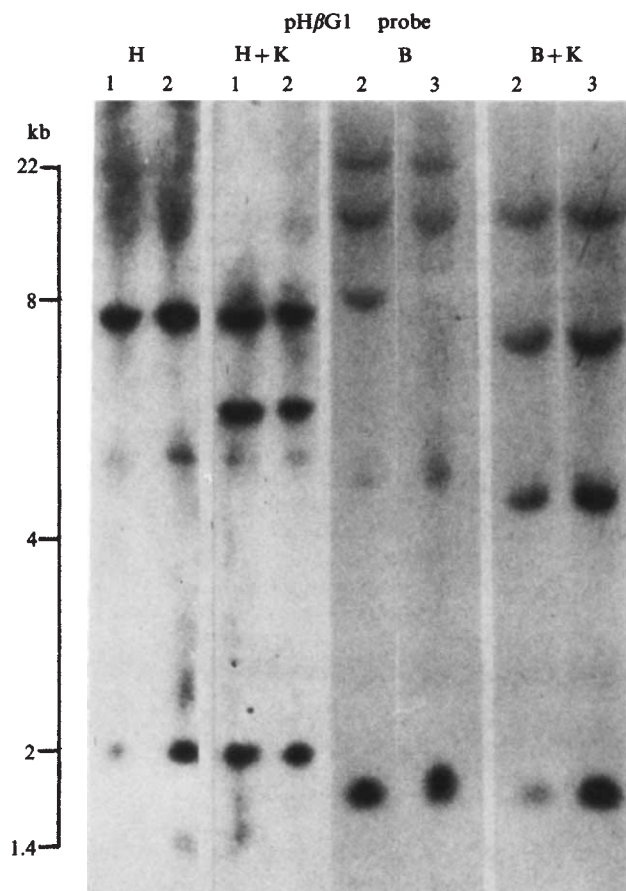
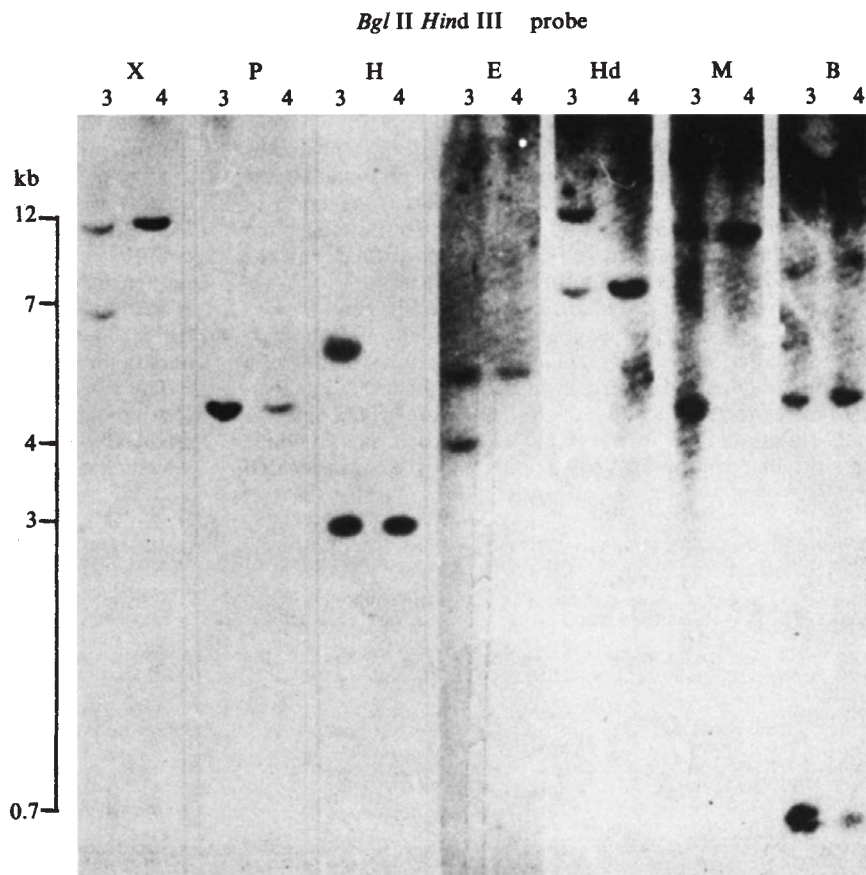


Fig. 3 Mapping the *KpnI* sites at the 3'-side of the β -globin gene in DNA from normal individuals and γ - β -thalassaemics: a 3' *KpnI* polymorphism. DNA from γ - β -thalassaemics (patient 3) or his normal spouse (no. 4) was digested with *KpnI* (K), *HpaI* (H) or *BamHI* (B) either single or in double digests. Only one-half of the 7.7-kilobase *HpaI* β -fragment is cleaved by *KpnI* to give the *HpaI* + *KpnI* double digest fragment of 5.5 kilobases; one half of the 22-kilobase 3' *BamHI* β -fragment is cleaved at the same *KpnI* site described previously⁸ to give the 4.6-kilobase double digest fragment and the other half lacks this site and is cleaved at a second *KpnI* site 3.1 kilobases further in the 3' direction. For abbreviations, see Fig. 1 legend.

Fig. 4 β -globin gene fragments in γ - β -thalassaemia, detected with the *Bgl*II-*Hind*III probe for the 5'-extragenic regions of the β -globin gene. Filter-bound DNA from a γ - β -thalassaemic patient and his normal spouse was prepared as described in Fig. 2 legend. The *Bgl*II-*Hind*III probe was prepared from a cloned *Hind*III fragment from the β -globin gene of a β^0 -thalassaemia patient (F. G. Grosveld, H. H. Dahl, E. De Boer and R.A.F. unpublished) by double digestion with these enzymes, followed by electrophoresis on a 1.2% agarose gel. The fragments were extracted according to the method of Tabak and Flavell²⁴ except that the DNA fragment was eluted from the hydroxyapatite with 400 μ l of 0.5 M EDTA (pH 7.5) instead of phosphate buffer. For abbreviations, see Fig. 1 legend.



The effect might be either *cis*- or *trans*-acting.

Second, it is possible that the reduction in β -globin gene expression is a result of the deletion mapped, but that its effect is not confined to the 'deleted' chromosome; it could be a general effect on both β -globin genes. To explain this, we could postulate that a *trans*-acting component is coded for by a gene in the $\gamma\delta$ -globin gene region. The fact that the β/α synthetic ratio is 0.49 instead of 1 is, however, more in line with the inactivation of one chromosome, presumably from the one carrying the deletion, by a *cis*-acting effect; a precise reduction of β -globin gene expression by a factor of two as a result of a drop in the level of a *trans*-acting component by the same factor seems unlikely, but this is not excluded by our data.

How do we explain the lack of β -globin gene activity by a *cis*-acting effect of the deletion? We could argue that the transcription unit of the β -globin gene extends into the DNA that has been deleted; conceivably the promoter or another regulatory sequence would be located more than 2.5 kilobases to the 5'-side of the β -globin gene. This is unlikely; a rabbit β -globin gene containing only 1.4 kilobases of the sequences upstream from the 5'-end of the gene is transcribed to give active globin mRNA when introduced into mouse L cells by transformation¹⁸, there is no reason to believe that the homologous human gene would do otherwise. We have also mapped the unspliced rabbit β -globin pre-mRNA to be a transcript of the mRNA coding regions plus the two intervening sequences; the 5'-extragenic sequences are not present on this transcript¹⁹.

The final explanation is that sequences far from the β -globin gene also influence its activity in *cis*. For example, a DNA sequence in the $\gamma\delta$ -globin gene region could be essential for β -globin gene activity. Specifically, we have considered the possibility that the $\gamma\delta\beta$ -region is split into chromosomal domains containing the fetal ($G\gamma$ and $A\gamma$) and adult (δ and β) globin genes, respectively¹². DNA sequences upstream from the δ -globin gene might be necessary to organise an active configuration for the whole $\delta\beta$ -region; their deletion would render this impossible and hence inactivate the β -globin gene,

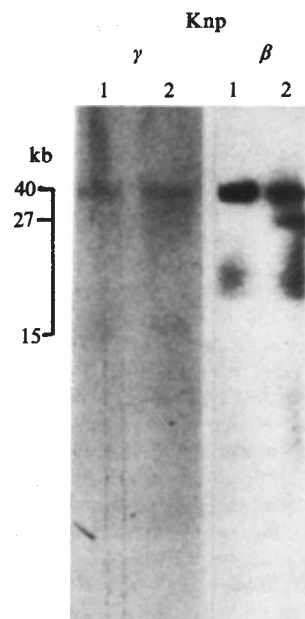


Fig. 5 the $\gamma\delta\beta$ -globin gene fragment in *Kpn*I digests of DNA from normal individuals and from γ - β -thalassaemics. DNA, 10 μ g, from patient 2 and his normal spouse (no. 1) was digested with *Kpn*I and electrophoresed on a 0.5% agarose gel for 72 h at 1 V cm^{-1} . The DNA was blotted, hybridised to either pH γ GI or pH β GI DNA and further processed as described in Fig. 3 legend. The γ -probe filter was washed only to $0.3 \times \text{SSC}$ to detect possible abnormal embryonic globin gene fragments. The faint 15-kilobase band contains the embryonic ϵ -genes and the 39-kilobase band contains the $G\gamma$ -, $A\gamma$ -, δ - and β -globin genes (see Fig. 1, ref. 8). Note that the 27-kilobase fragment in the DNA from the γ - β -thalassaemic patient only hybridises with the β -probe.

even though it would remain present on the chromosome. If these putative sequences were closely linked to the δ -globin gene and if they were essential for β -globin gene expression, the presence of the δ -globin gene in the population would be selected for by this linkage. This might explain why the apparently functionless δ -globin gene (which only contributes about 3% of the adult non- α -globin chains) is maintained in the human gene pool. It is also conceivable that some types of classical β -thalassaemia might be the result of mutations in the same DNA sequences discussed here, that is, further from the β -globin gene than has been hitherto considered.

Alternatively, the inactivation of the β -globin gene in γ - β -thalassaemia might be the result of a 'position effect' of the type previously described in *Drosophila*; here, the juxtaposition of a gene and a segment of heterochromatin results in the inactivation of the gene. Conceivably, the 5'-break point of the deletion in γ - β -thalassaemia is in a segment of heterochromatin which causes the inactivation of the β -globin gene when translocated

to a position next to the gene.

The existence of sequences influencing gene expression in eukaryotes over large distances has its precedents. The mapping of distal DNA sequences in the human β - and δ -globin gene regions, which influence the expression of the γ -globin genes, has recently been described by ourselves¹³ and by others^{6,7}. In the latter cases, however, the putative regulatory sequences repress γ -globin gene expression in adult life; their deletion results in the persistence of γ -globin gene expression. In the case described here, the opposite is seen, namely, regulatory sequences are required to activate β -globin gene expression. Hopefully, the analysis of other rare types of thalassaemia may further identify DNA regions involved in the expression of the human fetal and adult globin genes.

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LETTERS

Test of the inverse Compton model for Cygnus X-1

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The X-ray spectrum of Cygnus X-1 from ~ 1 keV to ~ 200 keV exhibits two distinct components. The spectrum below ~ 10 keV is soft and may be the tail of comptonised black body if the turnover at ~ 1 keV is real, while that above ~ 10 keV is a simple power law^{1,2}. The spectra above 200 keV remain uncertain with some showing a cutoff but others a continuation of the power law³. The power law hard spectrum is thought to be the result of unsaturated Compton up-scattering of soft photons by hot thermal electrons⁴. The comptonisation region may be the optically thin inner part of the disk or spherical accretion flow near the black hole or a hot corona surrounding a thin disk¹. The unsaturated Compton model is attractive as it accounts for the power law spectrum with index $\alpha \sim 1$ ($I_\nu \propto \nu^{-\alpha}$) and the alleged high electron temperature ($T_e > 10^9$ K). However, there are still questions about the origin of the soft photon supply and stability. As important conclusions are drawn from this model (for example, interpretation of the high energy cutoff as a measure of the electron temperature and α as a measure of the Kompaneet parameter $y \equiv (4kT_e/m_e c^2)\tau_{es}^2 \approx 4/(3\alpha + \alpha^2)$, $\tau_{es} \equiv$ electron scattering depth)^{4,5}, it is crucial to have independent verification of this idea. We propose here a more critical test and show that the hard X-ray data available to date strongly support this model.

A steady supply of soft photons of mean energy $h\nu_0$ and luminosity L_i injected into a comptonising medium of Kompaneet parameter y will emerge with luminosity $L \sim L_i e^y$

at least for $y \leq 1.5$ (unsaturated comptonisation, for saturated comptonisation $y \gg 1$ and $L \sim L_i 3kT_e/h\nu_0$; see refs 5, 6 for numerical results of intermediate regimes). L has two spectral components: a power law of total luminosity L_h for photons with many scatterings and a residual soft component of luminosity L_s for photons with few scatterings. Using photon number conservation one finds

$$L_s = L_i \left(1 - q \frac{h\nu_0}{kT_e} e^y \right) \left(1 - \frac{q h\nu_0}{kT_e} \right)^{-1};$$

$$L_h = L_i (e^y - 1) \left(1 - \frac{q h\nu_0}{kT_e} \right)^{-1}$$

where $q \sim 0(1)$ depends weakly on y and spectral details of L_i . Hence for $h\nu_0 \ll kT_e$, we have the important prediction

$$L_h/L_s = e^y - 1 \quad \text{for } y \leq 1.5 \quad (1)$$

We now consider whether the large body of spectral data of Cyg X-1 reported in the past 15 yr is consistent with equation (1), assuming that we can identify L_s with the 2-10 keV luminosity and L_h with the 10-200 keV luminosity. Whether the 2-10 keV luminosity itself involves some comptonisation of even softer photons with electrons of lower T_e and y should not affect this test. It is assumed in the above identification that the (highly uncertain) luminosity below ~ 1 keV originates from a spatially separate region and is unrelated to that above 10 keV.

The sharpest test of equation (1) comes from the 'transition' data when Cyg X-1 exhibited anticorrelated variations in soft and hard X-ray luminosities, with the pivoting point at ~ 10 keV. We can find published simultaneous soft and hard X-ray data for only three such events (March 1971, May 1975, November 1975-February 1976), although there must be others (see ref. 8). As crude estimates we simply extrapolate published spectra from 2 to 10 keV for L_s and from 10 to 200 for L_h .

Table 1 Comparison of observed luminosity ratios with inverse Compton model predictions for Cyg X-1

Transition (and refs)		Derived from published data*				L_h/L_s predicted from Compton model $= e^y - 1$ for $y \leq 1.5$ see ref. 5 for $y > 1.5$	$(L_h + L_s)/y^{1/2}$
		2-10 keV luminosity $L_s/1.1$ $\times 10^{36} \text{ erg s}^{-1}$ (with assumed distance of 2.5 kpc)	10-200 keV luminosity $L_h/1.1$ $\times 10^{36} \text{ erg s}^{-1}$	Kompaneet parameter y	L_h/L_s		
March 1971 ¹⁰	High	12	$\sim 19^\dagger$	$\sim 1.85^\dagger$	~ 1.6	~ 5.4	~ 22.8
	Low	5	35	2.58	7.0	7.6	24.9
May 1975 ^{11,12}	High	10	10	0.54	1.0	0.7	27.2
	Low	7	21	1.32	3.0	2.7	24.4
November 1975 to February 1976 ^{8,13,14}	High	$\sim 12^\ddagger$	9.5§	0.72	~ 0.8	1.0	~ 25.3
	Low	$\sim 7^\ddagger$	19.5§	1.19	~ 2.8	2.3	~ 24.3

* Errors for L_h and y listed in Table 2. Errors for L_s cannot be estimated.

† Large reported error.

‡ Only 3–6 keV band data available¹³, but they agree with extension of power law spectra from above 10 keV. Large error possible.

§ High state is average of refs 8 and 14. Low state is average of pre-November 1975 and post-February 1976 data of ref. 8

Table 2 Hard X-ray data for Cyg X-1 (1966–79)

Reported photon number spectrum $N_E = A(E/\text{keV})^{-\beta} \text{ cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$		10–200 keV luminosity		Observation date (and refs)
A	β	$L_h/1.1 \times 10^{36} \text{ erg s}^{-1}$	$y = \frac{4}{\beta^2 + \beta - 2}$	
(1) 3.58	1.93 ± 0.2	$14.0^{+16.8}_{-7.4}$	$1.09^{+0.38}_{-0.23}$	September 1966 ¹⁵
(2) $3.85^{+2.98}_{-1.71}$	1.75 ± 0.2	$30.5^{+7.7}_{-5.7}$	$1.42^{+0.63}_{-0.35}$	May 1967 ¹⁶
(3) 4.57 ± 0.61	1.91 ± 0.03	$19.3^{+5.3}_{-4.4}$	$1.12^{+0.05}_{-0.03}$	June 1969 ¹⁷
(4) 4.98	2.14 ± 0.2	$8.8^{+10.0}_{-4.5}$	$0.85^{+0.25}_{-0.16}$	May 1969 ¹⁸
(5) 3.54 ± 2.44	1.89 ± 0.22	$16.2^{+49.2}_{-14.0}$	$1.16^{+0.47}_{-0.28}$	April 1969 ¹⁹
(6) 5.41 ± 1.53	1.92 ± 0.10	$22.0^{+1.3}_{-2.7}$	$1.11^{+0.17}_{-0.13}$	April 1969 ¹⁹
(7) $1.32^{+2.0}_{-0.79}$	1.6 ± 0.4	$19.2^{+22.5}_{-9.2}$	$1.85^{+4.4}_{-0.85}$	March 1971 ¹⁰
(8) 1.29 ± 0.09	1.45 ± 0.03	$34.9^{+1.9}_{-2.0}$	$2.58^{+0.20}_{-0.18}$	March 1971 ¹⁰
(9) $1.44^{+0.60}_{-0.42}$	1.66 ± 0.15	$16.4^{+5.1}_{-3.6}$	$1.66^{+0.57}_{-0.36}$	October 1973 ²⁰
(10) $1.0^{+0.26}_{-0.20}$	1.51 ± 0.1	$21.1^{+4.5}_{-3.5}$	$2.23^{+0.63}_{-0.41}$	October 1974 ²⁰
(11) 4.4 ± 1.0	1.84 ± 0.05	$24.4^{+12.1}_{-8.9}$	$1.24^{+0.10}_{-0.08}$	July 1972 ²¹
(12) 8.5 ± 3.4	2.01 ± 0.09	$24.5^{+20.0}_{-14.0}$	1.0 ± 0.11	July 1972 ²¹
(13) $12.6^{+19.0}_{-7.6}$	2.4 ± 0.4	$8.7^{+6.3}_{-3.0}$	$0.65^{+0.35}_{-0.19}$	November 1975 ¹⁴
(14) $2.51^{+0.93}_{-1.46}$	1.7 ± 0.2	$24.3^{+10.4}_{-6.8}$	$1.54^{+0.76}_{-0.40}$	November 1975 ¹⁴
(15) $3.15^{+1.85}_{-1.16}$	1.8 ± 0.2	$20.5^{+8.4}_{-5.5}$	$1.32^{+0.53}_{-0.32}$	May 1975 ¹²
(16) $27.9^{+16.3}_{-10.3}$	2.6 ± 0.2	$9.7^{+2.5}_{-1.7}$	$0.54^{+0.11}_{-0.08}$	May 1975 ¹²
(17) 0.99 ± 0.43	1.53 ± 0.10	$19.1^{+22.4}_{-11.9}$	$2.14^{+0.56}_{-0.39}$	June 1975 ²²
(18) 1.85 ± 0.45	1.73 ± 0.06	$15.9^{+9.2}_{-6.4}$	$1.47^{+0.16}_{-0.13}$	July 1975 ²²
(19) 2.52 ± 0.69	1.81 ± 0.07	$15.8^{+10.7}_{-7.1}$	$1.30^{+0.15}_{-0.13}$	July 1975 ²²
(20) $7.5^{+7.62}_{-3.86}$	2.21 ± 0.27	$10.3^{+3.4}_{-2.4}$	$0.79^{+0.29}_{-0.19}$	November 1975 ⁸
(21) $3.1^{+1.80}_{-1.21}$	1.87 ± 0.17	$15.3^{+3.0}_{-2.7}$	$1.19^{+0.35}_{-0.24}$	November 1975 ⁸
(22) $4.7^{+1.70}_{-2.65}$	1.85 ± 0.15	$25.1^{+4.0}_{-3.9}$	$1.22^{+0.32}_{-0.23}$	November 1976 ⁸
(23) $4.9^{+1.70}_{-2.54}$	1.88 ± 0.15	$23.3^{+4.2}_{-3.4}$	$1.17^{+0.30}_{-0.21}$	November 1976 ⁸
(24) $6.1^{+2.67}_{-1.87}$	1.89 ± 0.13	$27.9^{+4.3}_{-3.5}$	$1.16^{+0.24}_{-0.18}$	October 1977 ⁸
(25) $5.1^{+1.39}_{-1.14}$	1.83 ± 0.09	$29.5^{+3.2}_{-3.1}$	$1.26^{+0.19}_{-0.15}$	November 1977 ⁸
(26) $0.92^{+0.54}_{-0.34}$	1.42 ± 0.2	$28.2^{+13.7}_{-8.6}$	$2.78^{+2.87}_{-1.00}$	September 1977 ²³
(27) $2.06^{+1.21}_{-0.76}$	1.6 ± 0.1	$29.9^{+41.9}_{-17.3}$	$1.85^{+0.44}_{-0.26}$	December 1977*

* P. Nolan, unpublished HEAO 1 data.

Table 1 lists the data for the transitions. Despite expected errors they show surprising agreement with theory except for the (soft) 'high' state before the 1971 transition, whose large reported error box is still consistent with the inverse Compton model. Such agreement also strongly suggests that most of the 2–10 keV soft photons have the same primary origin as the power law componentised photons > 10 keV and do not come from a spatially detached region.

However, six data points are far from conclusive. We have selected 27 reported spectral measurements of hard X rays in the range ~ 20 – ≤ 200 keV with acceptable reported or apparent error margins ($\leq 30\%$ in α , see Table 2). Again we extrapolate all power law spectra down to 10 keV and up to 200 keV to obtain a uniform definition of L_h . Figure 1 plots observed L_h versus y . From equation (1) we expect an overall rise of L_h with y despite intrinsic variations in L_s . This is indeed borne out in Fig. 1. Of course, these data all have large experimental errors as well as detector sensitivity and limit differences. But any systematic bias is likely to be washed out by the large number of independent measurements. The qualitative increase of L_h with y in Fig. 1 is probably genuine and consistent with the inverse Compton model.

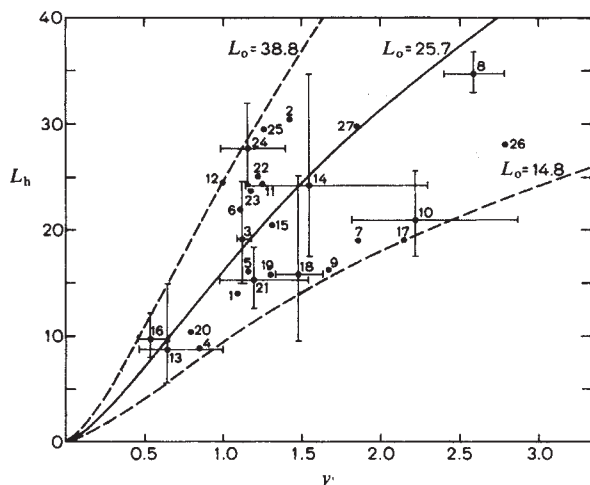


Fig. 1 Plot of L_h versus y listed in Table 2. Only a few sample error boxes are included for illustration. The solid curve represents weighted least square fit with function in equation (3). The dashed curves correspond to extremal values of L_0 sandwiching all data points. Numbers correspond to those in Table 2.

A closer look at the transition data also reveals that the variation of the combined luminosity from 2 to 200 keV is definitely correlated with y . From the limited data (Table 1 last column) we find empirically

$$(L_s + L_h) \cdot y^{-1/2} \approx \text{constant} \equiv L_0 \quad (2)$$

This behaviour is consistent with the theoretical prediction that the accretion rate of a thin disk $\dot{M}$ onto a black hole varies as $\dot{M} \sim \text{constant} \times y^{1/2}$ because of the transonic nature of the radial drift. This result is discussed in detail elsewhere⁹. If equation (2) is valid universally then we should have (see equation (1))

$$L_h = L_0 y^{1/2} (1 - e^{-y}), \quad L_0 \approx \text{constant for } y \leq 1.5 \quad (3)$$

A least square fit of the data with this function gives $L_0 = 25.7$ (Fig. 1). More significantly, all data points lie within a narrow wedge corresponding to $14.8 \leq L_0 \leq 38.8$ despite the wide range of L_h and y (Fig. 1).

Because of the crude nature of our data analysis and lack of calibration standard for the measurements made by different groups at different times, the above results must be viewed with extreme caution. Despite such uncertainties, the data suggest that, at least qualitatively, (1) predictions of the inverse Compton model is verified, (2) (2–200 keV luminosity L)/ $y^{1/2}$

varies only slightly despite large changes in L and y . We urge that future observations should try to monitor carefully the long term variation of L_s and L_h with y simultaneously.

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Solar oscillations, stellar oscillations and cosmology

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Two implications of the recent observations¹ of low angular, high radial overtones of the whole Sun are reported here. The first implication is that other main sequence stars are likely to be oscillating in similar modes and that precision spectroscopy as well as photometry from space is capable of detecting these oscillations, thereby extending seismology to stars. The second implication is a solar helium abundance $Y \approx 0.17$ with implications for cosmological models.

Recent observations¹ using the 769.9 nm resonance line of potassium of velocity oscillations of the whole disk of the Sun have resolved the broad 5-min oscillation peak into over 20 discrete spectral lines of amplitudes between 0.1 and 0.3 m s⁻¹ and a nearly equal spacing of 67.80 ± 0.15 μ Hz. It can be readily shown that such global velocity studies could be extended to the brighter stars using large telescopes as flux collectors and such attempts have been already made². Extension to fainter stars is likely to be possible using precision differential photometry from above the turbulence and variable transmission of the terrestrial atmosphere. For radial modes a rough measure of the expected photometric amplitude for a given velocity amplitude is provided³ by $\Delta m_B \approx \Delta V/40$ where Δm_B is the blue magnitude amplitude and ΔV is the velocity amplitude in km s⁻¹. Thus for the Sun the quoted velocity amplitudes of individual frequency components imply fractional variations of 6×10^{-6} to 2×10^{-5} . Such precision has not as yet been achieved with ground-based telescopes but statistically such variations are apparently detectable at the 3σ level if more than 3×10^{11} and 3×10^{10} photons are counted or the equivalent charge is digitised. Such light fluxes are within reach of medium telescopes for stars brighter than 6 mag. Clearly, differential measurements on at

least three stars will have to be made simultaneously to provide sufficient differential systematic stability and redundancy to identify the variable star from its comparison stars. Were the equivalent of the structure of the 5-min oscillation of the Sun to be found in stars a powerful tool of analysis of main sequence stars would become available.

The second implication follows from the interpretation of the mean spacing of 67.80 μHz with alternate $l = 0$ and $l = 1$ modes, of increasing radial order within the context of the standard model of the solar interior⁴ or a model with a surface enhanced heavy element abundance⁵. Both models, if they are to agree with these observations, imply a mean heavy element abundance $Z \leq 0.004$ and a low solar neutrino flux ≤ 2.3 SNU, consistent within two standard deviations of the results of the most recent measurements⁶. The same observations and interpretation lead to a solar helium abundance of $Y \leq 0.17$, a value which agrees with that of the quiet solar wind⁷. It was already realised some 10 yr ago⁸ that such a low helium abundance, among other possibilities, would lead to a solar neutrino flux consistent with the low values observed.

Thus three solar phenomena, namely oscillations, the particle wind and the neutrino flux are consistent with $Y \leq 0.17$. As hydrogen burning during the lifetime of the Sun would have increased the helium concentration and no processes are known which could have destroyed helium in the Sun, it follows that the helium abundance of the primeval material out of which the Sun formed was ≤ 0.17 . This value is substantially below the mean of

the values for Y obtained from other observations relating to the solar atmosphere or other objects in the Universe⁹. It is also substantially below the preferred value of current simple cosmological models¹⁰⁻¹² of some 0.25–0.27 even if one corrects the expectations for the new value of the neutron mean life¹³. It is possible that this model can be reconciled with a lower helium abundance by, for example, requiring sufficiently degenerate neutrinos¹⁰⁻¹².

A more unambiguous determination of the helium abundance of the deep solar interior may result from definitive observation and identification of the fundamental and low order radial modes of the Sun¹⁴.

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METEOSAT observations of diurnal variation of radiation budget parameters

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Preliminary estimates of the outgoing IR flux and albedo obtained at hourly intervals, during one day, using digital METEOSAT data are reported here. The diurnal variation of these parameters varied according to the surface or cloud type over which they were being measured. The albedo over the desert, and both high and low clouds, remained constant, but altered over the sea surface. The emitted IR flux was constant over sea surfaces and low clouds, but altered over the desert during the day, due to surface heating. These diurnal variations can introduce biasing errors into time averaged radiation budget parameters, derived from polar orbiter data.

Radiation budget measurements of the Earth's atmosphere/surface system can provide information about the driving mechanisms of the Earth's atmospheric and oceanic circulation systems. Until recently, global radiation budget measurements have only been made from satellites in polar orbits^{1-4,9} which sample specific areas of the Earth approximately once every 12 h. Because of the long time interval between samples and the restriction that these parameters are measured for approximately the same solar zenith angle, various assumptions have to be made when calculating daily mean values from these measurements.

The planetary albedo is determined by comparing the reflected solar radiation (measured from the satellite) with the incident solar energy calculated from the solar constant. The daily mean is inferred from one single daylight measurement by assuming the albedo is not dependent on solar zenith angle and that the radiation is scattered isotropically from all surfaces. Both of these assumptions are not valid in certain cases, such as for large solar zenith angles over the poles and for 'sunglint' reflection from the ocean surface. Sunglint occurs on the part of

Table 1 The diurnal mean radiation budget parameters estimated from Polar Orbiter and Geosynchronous Orbiter data (W m^{-2})

Surface/cloud type of target area	Location of target area	Actual mean calculated from hourly METEOSAT values Outgoing flux ($\pm 30^* \text{ W m}^{-2}$)	METEOSAT value at 0900 h	% Difference of 0900 h value from diurnal mean
Over desert	26° N 14° W	319	328	+3%
Over ocean	26° N 15° W	315	314	+0%
Over cumulus cloud	15° S 6° W	300	301	+0%
Over thick cloud extending up to the tropopause	26° S 35° E	214	200	-7%
Albedo (± 0.05)				
Over desert	26° N 14° W	0.32	0.32	0%
Over ocean	26° N 15° W	0.09	0.07	-22%
Over cumulus cloud	15° S 6° W	0.39	0.47	+21%
Over thick cloud extending up to the tropopause	26° S 35° E	0.59	0.62	+5%
Net flux ($\pm 50 \text{ W m}^{-2}$)				
Over desert	26° N 14° W	-114	-122	7%
Over ocean	26° N 15° W	-42	-42	0%
Over cumulus cloud	15° S 6° W	-53	-58	9%
Over thick cloud extending up to the tropopause	26° S 35° E	-49	-23	53%

* The uncertainties refer to the absolute value, not to the random errors.

the Earth's surface where direct reflection from the Sun is possible as viewed by the satellite.

The outgoing long-wave radiation will have a diurnal variation over cloud-free landmasses as the ground will be heated by the incident solar radiation during the day, increasing the emitted flux. Hence twice-daily measurements from polar orbiters will not give an accurate daily mean.

Data from satellites in geosynchronous orbits such as METEOSAT are now available. These satellites are at a fixed

point above the Equator (0° lat and 0° long for METEOSAT) and record images of the entire Earth's disk once every 30 min. This allows the radiation budget parameters to be adequately sampled for an investigation into their diurnal variation. The images are at three wavelengths, visible ($0.6\text{--}0.9\ \mu\text{m}$), IR ($10.8\text{--}12.2\ \mu\text{m}$) and water vapour ($5.8\text{--}7.2\ \mu\text{m}$). The visible and IR channels were used to determine the albedo and long-wave flux respectively. Initially four target areas were chosen with different surface/cloud types which would be expected to have differing diurnal radiative properties. These areas were $150\ \text{km}$ square on the Earth's surface which represents an average over at least 650 pixel values. The four areas chosen are described in Table 1, and the two cloud-free areas are shown in Fig. 1.

The visible channel has no radiometric calibration although attempts are being made to provide one⁵. For this study, a calibration was obtained by assuming the planetary (or system) albedo over North-west Africa to be 0.26 (ref. 6) at local noon on 21 August 1978. This value was based on aircraft and surface measurements taken over the area on 4 September 1974. The seasonal variation in surface albedo noted by Rockwood and Cox⁶ was further south than the area chosen for the METEOSAT measurements. However, a check was made by comparing planetary albedos measured from METEOSAT at local noon in April, August and November and no significant change in albedo was noted for these months. Knowing the incident solar radiation for the 21 August, and assuming that the surface/atmosphere reflects radiation isotropically, the radiometer responses can be calibrated in terms of reflected energy. The METEOSAT visible radiometer scales linearly with incident radiation intensity, so having obtained a calibration for one radiometer value, the planetary albedo for different areas can be estimated. When the data become available, it is hoped to improve this calibration technique by a direct comparison of METEOSAT images with NIMBUS 7 ERB data and GOES EAST (70°E , 0°N) data where the images overlap.

The long-wave flux was derived from the calibration given by ESA⁷ which was based on a comparison between the spacecraft digital counts and measured sea-surface temperatures. An atmospheric transmission model used for the NOAA satellite data¹ converted the $11\text{-}\mu\text{m}$ radiance to total outgoing flux at the top of the atmosphere. Figure 2 shows how the albedo and outgoing flux varied during one day (5 November 1978) for each

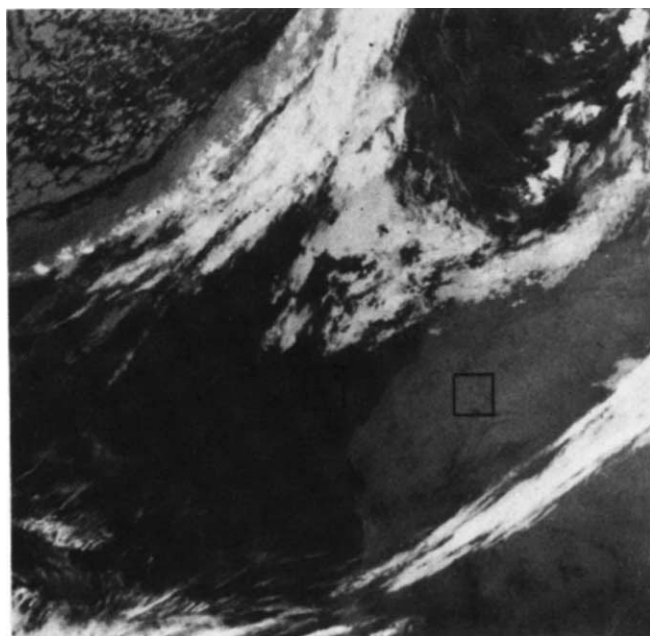


Fig. 1 The two cloud free target areas over NW Africa selected for the measurement of radiation budget parameters.

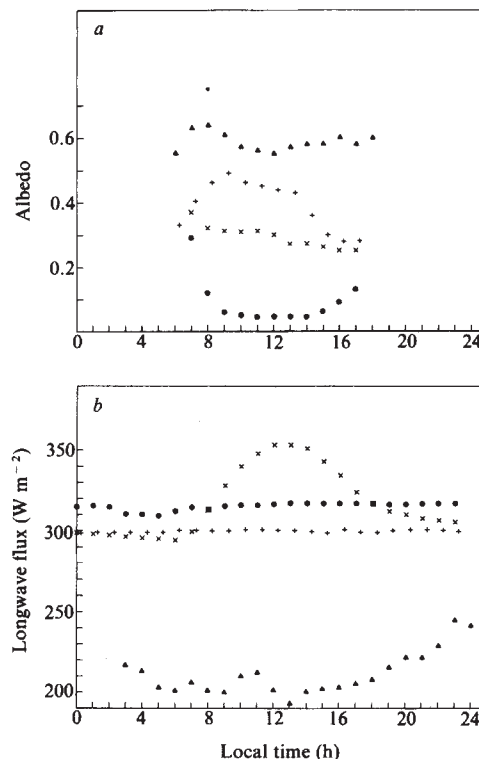


Fig. 2 The diurnal variation of albedo (a) and outgoing flux (b) as measured by METEOSAT over four surface/cloud types. $\times$, Over land; $\bullet$, over sea; $+$, over low Cu cloud; $\blacktriangle$, over high CuNb cloud. The absolute values of albedo and long-wave flux have an uncertainty of 0.05 and $30\ \text{W m}^{-2}$ but the relative uncertainties will be much smaller.

target area. The albedo remained fairly constant over the desert, but over the sea it increased markedly with increasing solar zenith angle. This is because the sea surface can be relatively smooth and so reflects more radiation at glancing angles⁸. In contrast, cumulus cloud has a vertical structure which will reflect more of the radiation at glancing angles back in the direction from which it came. The dense high cloud gave a variable albedo which was due to changes in its structure during the day. The long-wave flux over the land increased markedly around noon due to surface heating, whereas over the sea and low cloud, the flux remained essentially constant. For high level cloud, the outgoing flux was variable which would be due to changes in cloud top altitude and structure.

Table 1 illustrates the biasing errors, which can result from one single measurement taken from a polar orbiter; actual diurnal means are compared with the instantaneous 0900 h value using the METEOSAT observations. The latter value would correspond to the measurement made by a polar orbiter such as the NOAA series which passed over at a local time of 0900 h. The errors in the parameters, derived from the NOAA satellites, varied according to surface/cloud type. Long-wave flux errors are small and, as they only become significant over hot deserts, they are unlikely to affect the calculated global long-wave flux significantly. The albedo errors are more significant, especially as large areas of the globe are covered by ocean and low cloud; hence global albedos from polar orbiters would have a biasing error. To correct polar orbiter data sets, such as the one described by Gruber and Winston⁹, the parameters over the ocean and low cloud should be corrected by the percentage differences listed in Table 1.

Also listed is the net flux which, being the small difference between two fluxes, has a large uncertainty. The errors are still present, although over the ocean, the errors are reduced because the albedo and outgoing flux errors are of opposite sign.

These results, taken from one day of METEOSAT data, show that biasing errors do exist in radiation budget parameters,

obtained from polar orbiters. The geosynchronous satellite data, which are now available, allow correction factors to be determined when calculating diurnal, monthly and annual means from polar orbiter data.

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Fossil charcoal as evidence of past atmospheric composition

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The presence of charcoal in the geological record offers a new approach to assessing oxygen levels in palaeoatmospheres. Charcoal is formed by the incomplete combustion of woody tissues. The combustion of wood is supported by the generation of the combustible gases carbon monoxide and methane during the pyrolysis process. As the combustion ranges of these gases are restricted with decreasing oxygen availability to a level below which no combustion will occur, the production of charcoal and its appearance in the geological column constitute a record of changing oxygen availability through time. We show here that the occurrence of charcoal in rocks from the Lower Carboniferous onwards suggests that atmospheric oxygen never fell below 0.3 of present atmospheric level (PAL) during this time. Detrital charcoal formed by forest fire must have contributed an appreciable fraction to inert carbonaceous matter in sediments (kerogen) during post-Devonian time.

Natural fires have been documented in grassland, tundra, boreal forest, deciduous forest and tropical evergreen forest ecosystems in North America^{1,2}. In the southeastern US, fires are common in drought-dried peat³. Burning may also occur in dried strand-line accumulations of both aquatic and land-derived vegetation⁴.

The fossil record of terrestrial plant life indicates that small, vascular plants with upright stems had colonised the land surface by the late Silurian (~400 Myr) and that by the close of the early Devonian there were land plants at least 1 m in height and 50 mm in diameter⁵. Such plants would have been capable of forming what could loosely be called reed-swamp, or brush vegetation in drier habitats. In conditions of drought such vegetation would presumably have been capable of supporting a fire, with associated charcoal production. By late Devonian time (~350 Myr) we have records of forest trees in excess of 1 m in diameter; at this stage, forest fires, comparable with those of the present day, would have been possible. We have no evidence of terrestrial vegetation capable of fuelling any natural fire before the close of the Silurian, but presumably, before the evolution of upright terrestrial plants, accumulations of aquatic plant (algal) debris on strand lines may have presented suitable fuel for naturally occurring fire. Sizeable algae, which could accumulate on a strand line, are known from the Ordovician onwards⁶

(~500 Myr). However, such strand-line accumulations of algal material would surely not ignite readily by lightning strike, and we know of no published record of charcoal of pre-Devonian age.

Charcoalified plant fragments are locally abundant in many post-Devonian sedimentary sequences. They may be identified by silky lustre and friability in hand specimen and are characterised by high reflectance in polished rock sections. Such material is known as fusinite⁷; accumulations of fusinite clasts make up the coal macrolithotype fusain⁸. Fusinite clasts are typically fragments of wood, showing well preserved cellular structure, although more rarely leaves and other plant organs can be preserved in this way^{9,10}. Certain features of cell wall structure revealed by scanning electron microscope examination are particularly characteristic of a pyrolysis origin¹¹. Fine anatomical details of pits and wall thickenings are preserved in charred, woody tissues, but the original components of the cell wall (middle lamella, primary wall and secondary wall) lose their intrinsic identity on pyrolysis. This apparent homogenisation of the wall takes place above 300 °C and is observed in all charcoals over a wide range of geological ages (see Fig. 1). With normal coalification of woody tissue (that is, incorporated in sediment without previous charring) the middle lamella is still visible in structured plant material up to medium volatile bituminous rank of coal¹².

Charcoal results from incomplete combustion of plant tissues¹³ and is a common product of natural wildfires (forest fires)¹⁴. Such burning has three essential requirements: (1) a suitable fuel, (2) an ignition source, and (3) the necessary level of atmospheric oxygen to support combustion. Suitable natural ignition sources are lightning strike, volcanic activity, meteorite fall, spontaneous combustion and sparks due to rock fall. Of these, lightning strike is the most important reported cause of naturally ignited fires. Some two-thirds of all forest fires along the west coast of the US may be attributed to this cause, and 1–2% of fires in the Florida Everglades are similarly ignited¹⁵. The frequency of lightning discharges is estimated at 100 s⁻¹ over the surface of the Earth¹⁶. Direct evidence of lightning strike in the geological record is rare. However, Komarek¹⁷ notes that a petrified specimen of *Taxodium* sp. from the Miocene of Florida displayed a typical lightning streak along its length. Fulgurites result from the fusion of siliceous material (quartz sands) by lightning discharge. They are common in present day desert areas¹⁷ and are also known from older sediments¹⁸.

The pyrolysis of wood associated with its combustion proceeds by the evolution of water, carbon dioxide, formic acid and

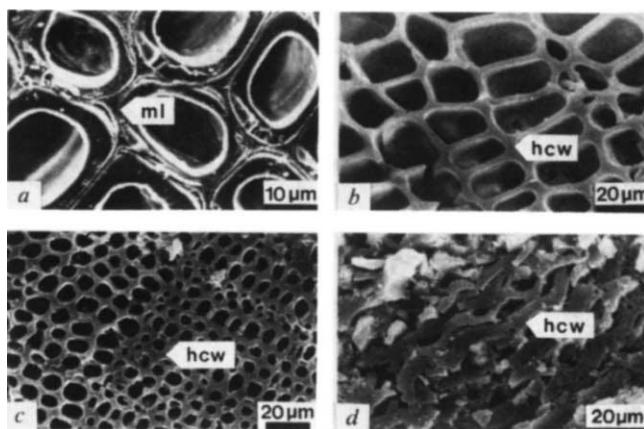


Fig. 1 Scanning electron micrographs of wood and charcoals. *a*, *Pinus sylvestris* wood showing the presence of the middle lamella between adjacent cells (ml) (scale bar, 10 µm). *b*, *c* and *d* (scale bars, 20 µm), fossil charcoals (fusinites) from the Tertiary Niedersächsische Braunkohle, Jurassic Scalby Formation and Carboniferous Coal Measures respectively showing homogenisation of the cell wall (hcw) without a visible middle lamella.

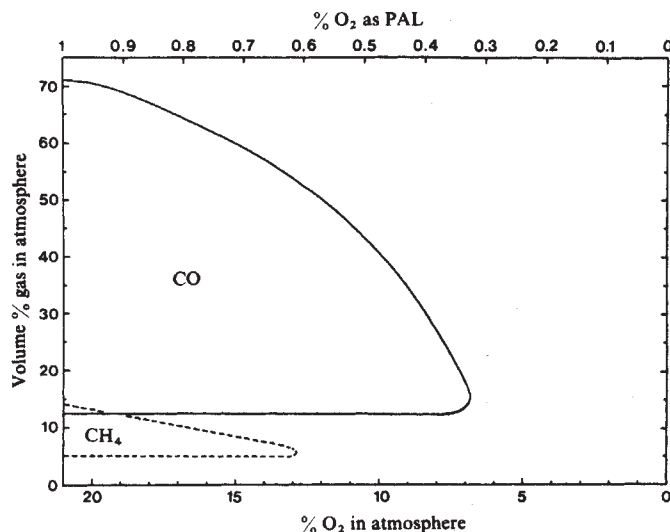


Fig. 2 Combustion ranges of carbon monoxide and methane for varying percentages of oxygen in the atmosphere (data taken from Coward and Jones¹⁹). As the combustion of these gases accounts for the burning associated with forest fire it is suggested here that the latter could not occur with $\leq 7\%$ oxygen in the atmosphere; this corresponds to ~ 0.3 PAL.

acetic acid with increasing temperature up to 280°C (ref. 13). Above this temperature, the combustible gases carbon monoxide and methane are released; it is the combustion of these gases which supports the flames constituting burning. The combustion ranges of carbon monoxide and methane are clearly defined with respect to the percentage by volume of combustible gas to air, and the limiting oxygen percentage in the atmosphere (see Fig. 2). Data from Coward and Jones¹⁹ for combustion of various gases in air diluted by nitrogen indicate that combustion of carbon monoxide will not take place in an atmosphere with $< 6.8\%$ oxygen; the corresponding figure for methane is 12.8% . It is also shown that with decreasing oxygen content of the atmosphere, the combustion range of each gas becomes more restricted. This means that at any stage of atmospheric evolution the occurrence of fire would have been limited by a threshold of oxygen availability.

Oxygen is believed to have been absent from the Earth's early atmosphere. The absence of oxygen in volcanic emissions has

led to the suggestion that the accumulation of oxygen in the early atmosphere has been either by photodissociation of water in the upper atmosphere²⁰ or from photosynthesis by plants. Proponents of both theories recognise that oxygenic photosynthesis had become the most important oxygen producer by Phanerozoic time. Berkner and Marshall²¹, favouring an initial photosynthetic build up, see a progression from 0.01 PAL of oxygen in the Cambrian to 0.1 PAL in the Silurian, and to more than PAL with the appearance of abundant land plant life in the Devonian and Carboniferous. Other workers place less emphasis on the contribution of oxygen by land plants, and stress the importance of the phytoplankton contribution. Tappan²² sees oxygen levels from this source as ~ 0.3 PAL in the Cambrian, more than PAL from Ordovician to Devonian, < 0.1 PAL in the Permo-Triassic and fluctuating above and below PAL for the rest of the Phanerozoic. Estimates of oxygen levels in the Phanerozoic of Berkner and Marshall, and of Tappan, are shown in Fig. 3 together with base lines for carbon monoxide and methane combustion. (The limiting oxygen percentages for combustion of carbon monoxide and methane can be seen to correspond to ~ 0.3 PAL and 0.6 PAL respectively.) The carbon monoxide combustion threshold is necessarily the base line for natural burning, and therefore, charcoal production. Based on Tappan's estimate, charcoal could not have been produced from late Carboniferous to early Jurassic times. However, fusain bands and lenses are widely distributed in Northern Hemisphere Carboniferous bituminous coals²³, and in northern Europe there is abundant clastic fusinite in Rhaeto-Liassic and Middle Jurassic sediments²⁴⁻²⁶. Clearly, Tappan's estimates of oxygen levels based on phytoplankton production are incompatible with this evidence, while the Berkner-Marshall oxygen curve is above the carbon monoxide combustion threshold from the mid-Devonian onwards, and is, therefore, consistent with the geological record of fusinite. A different view is adopted by Cloud²⁷, whose estimate of oxygen levels is based on an assumption of steady accumulation of photosynthetic oxygen throughout geological time. Cloud argues that the Pasteur point (0.01 PAL) was attained early in the Earth's history ($\sim 2,000$ Myr) and that levels of 0.1 PAL were reached in the late Precambrian. From his data the carbon monoxide combustion threshold would have been achieved by the late Cambrian, which is consistent with the geological record of fusinite.

The presence of the first charcoal in the geological record is an indication that oxygen levels in the atmosphere were above 0.3 PAL. The oldest sedimentary occurrences of charcoal largely in a non-carbonaceous matrix that we know of are in Lower Carboniferous strata from Donegal, Eire²⁸, and Fife,

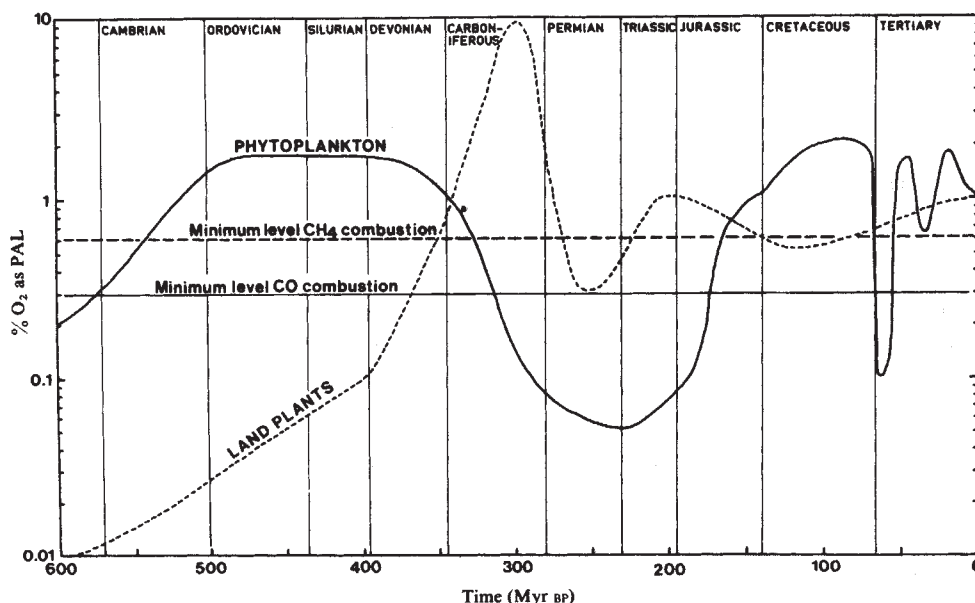


Fig. 3 Estimates of the level of oxygen in the atmosphere during Phanerozoic time based on the assumption that photosynthesis by phytoplankton was the main controlling factor²² (solid line) and corresponding hypothetical levels assuming land plant photosynthesis to be the main controlling factor²¹ (broken line). The estimate of Cloud²⁷ based on a steady build-up of oxygen level from all photosynthetic sources is not shown for reasons of clarity; this gives oxygen levels above 0.1 PAL at the start of the Phanerozoic and above 0.3 PAL by the late Cambrian, with a subsequent steady increase to PAL during the remainder of the Phanerozoic. The combustion thresholds for carbon monoxide and methane¹⁹ which necessarily limit the occurrence of forest fire burning, are also shown.

Scotland. The oldest documented record of charcoal in a carbonaceous matrix is from a transitional Devonian–Carboniferous ‘coal’ from Pennsylvania²⁹. In the present state of knowledge, it can therefore be argued that levels of oxygen greater than 0.3 PAL were attained at least by the latest Devonian and were subsequently maintained above this level. Thus the recognition of charcoal in sediments offers a useful quantitative indicator of the oxygen content of the atmosphere from the Devonian onwards.

Charcoal has a particular significance in another respect as a constituent of sedimentary rocks. Its inert character renders it highly resistant to microbiological or chemical degradation during sedimentation and subsequent diagenesis, while its thermal stability enables it to withstand high palaeotemperature of deep burial. It follows that from Devonian time onwards, charcoal could have made a significant contribution to the insoluble dispersed organic matter in sediments (kerogen). The total amount of kerogen is estimated to be some 500 times greater than all known coal deposits³⁰. However, because of its dispersed nature, this vast reservoir of carbonaceous material is unlikely to be exploited despite the ever increasing need for new fuel sources. Natural burning produces air-borne carbon particles³¹ and releases charcoal clasts at the site of fires¹⁴. Such inert carbonaceous products can contribute to kerogen. Air-borne carbon particulates are recognised in modern and Tertiary marine sediments^{31,32}, and structured fusinite micro-clasts occur in kerogen isolated from ancient sediments³³. A study of these kerogen particulates in post-Devonian sediments may help to establish the geographical and temporal distribution of pyrolysis-derived components and thus reveal a record of past forest-fire activity.

Charcoal has been largely overlooked or ignored by both sedimentologists and palaeobotanists in sediments of all ages (except, perhaps, coals); in view of the geochemical and palaeoecological significance of its occurrence, attention could usefully be concentrated on the first appearance of charcoal in the geological record. We would be particularly interested to learn of any charcoal occurrences of Devonian or earlier age.

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Stable isotope and sea-level data from New Guinea supports Antarctic ice-surge theory of ice ages

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Two theories of glaciation have received considerable attention, the Milankovitch orbital theory^{1,2} in which climatic change is ascribed to latitude-dependent variations in solar radiation that accompany changes in the Earth's orbital parameters, and the Antarctic surge hypothesis^{3–6}, in which a large Antarctic ice sheet ‘surges’ into the Southern Ocean thereby increasing the Earth's albedo, and the resultant cooling triggers the growth of ice in the Northern Hemisphere. The interval which encompasses the peaks of the last interglacial maximum at ~125 kyr (ref. 7) and its subsequent decline, with cooling of the North Atlantic⁸ and rapid fall of sea level^{9,10}, presumably also includes the event that triggered the succeeding ice age. The raised coral reefs of Huon Peninsula, Papua New Guinea, contain a particularly good record of the interval 140–105 kyr (ref. 11), from which we present oxygen isotope and sea-level data which seem to require an Antarctic surge at 120 kyr, and which also have a bearing on the role of the Milankovitch factor.

The structure of Huon Peninsula reefs VIIa, VIIb and VI is shown in Fig. 1. Relative to the rising landmass, the essential sea-level changes represented by these reefs are^{10,11}: (1) sea-level rise during building of reef VIIa; (2) minor fall during erosion at X in Fig. 1; (3) rise of >8 m during building of VIIb crest; (4) falling sea level, with continued growth of the patch-reef at Y during early stages; this regression eventually reached the base of reef VI; and (5) rise during building of reef VI. The envelope curve of sea-level changes interpreted from these and comparable reefs in Timor⁹ is shown in Fig. 2a; the tectonic uplift factor has been subtracted^{11–13}. Chronology is based on ²³⁰Th/²³⁴U determinations of these reefs^{12,13}, correlated with dated reefs in Timor where VIIa, VIIb equivalents are separated clearly⁹. Correlation extends to well dated reefs in Hawaii¹⁴ which contains two units believed equivalent to VIIa, VIIb on the grounds that each reef-building phase essentially is controlled eustatically⁹. Pooling the ²³⁰Th/²³⁴U data puts VIIa at 133 ± 4 kyr and VIIb at 120 ± 3 kyr (ref. 9). Reef VI dates at 105 kyr and correlates with similar reefs in Timor⁹ and Barbados¹⁵. The duration of building and emergence of VIIb are important in our subsequent discussion. First, between erosion unconformity X in Fig. 1 and the VIIb crest there is >8 m of

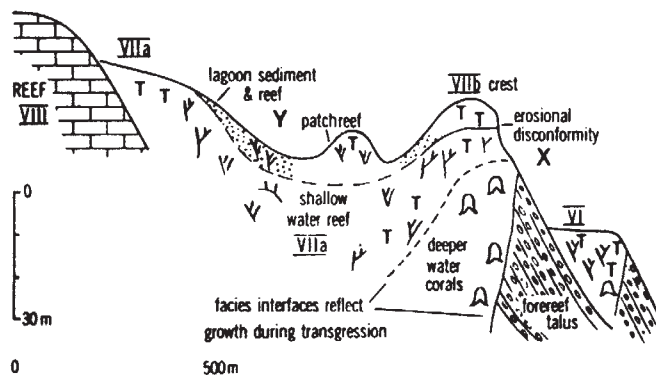


Fig. 1 Section of New Guinea reefs VIIa, VIIb, VI generalised from three separate sections (Gitua, Sazum, Sialum¹¹). Sampling points shown by *Tridacna gigas* symbol (T).

shallow water reef, representing 1–2 kyr of growth, on the basis of ^{14}C study of Holocene reef growth at the Huon Peninsula¹⁶. Second, between emergence of VIIb crest and cessation of coral lagoon–reef growth at points Y in Fig. 1, ~3 kyr minimum must have elapsed assuming both tectonic and glacio–eustatic factors acted at maximum known rates^{10,11}.

Stable isotope analyses from these reefs were based on the giant clam *Tridacna gigas*, for the following reasons. (1) Coral recrystallisation in the raised reefs is extensive and diagenetic exchange of carbon has been confirmed by ^{14}C analysis rendering corals unsuitable¹⁷. (2) Hermatypic corals fractionate stable isotopes in a manner related to growth habitat^{18,19}. (3) The massive aragonite shell of *Tridacna gigas*, readily checked for diagenesis by microscope and X-ray diffraction methods, is shown by ^{14}C generally to remain immune from carbon exchange and diagenesis¹⁷. (4) Most importantly, our analyses of modern specimens indicate temperature-controlled isotopic equilibrium with surrounding seawater. Table 1 lists $\delta^{18}\text{O}$ for 23 determinations from seven modern specimens as $-1.54 \pm 0.19\%$ PDB. The adjacent ocean yields -0.07% SMOW; using Epstein's²⁰ palaeotemperature equation plus 0.6% aragonite enrichment factor²¹ gives an isotopic temperature of 27 °C, which agrees with measured sea-surface temperatures in the area.

Stable isotope data from each reef phase are given in Table 1, together with estimated sea-level and mean age data. Multiple samples (numbers as given) were collected in each reef zone and multiple determinations made of each. The procedure was as follows: to measure the environmental average it is necessary to sample the full growth-width of a *Tridacna*, as stable isotope analysis of single growth lamellae in modern specimens clearly shows season-dependent variation²². Accordingly, the massive hinge region was sliced radially and each slab scrutinised for algal borings and calcite veins. Flawed samples were rejected. A thin strip extending across each slab was crushed, X-rayed to ensure aragonite purity, and the powder roasted for 30 min at 400 °C to eliminate any possible organic shell material^{20,23}. Extraction of CO_2 follows McCrea²⁴, and mass spectrometry was done on a modified MS-12 machine²⁵. Instrument precision is $\pm 0.05\%$, reproducibility including chemistry is $\pm 0.1\%$.

Almost all samples grew in reef sites exposed to the open ocean, either in forereef or shallow reef crest positions. The only exceptions are those from the regression phase of VIIb (Table 1), which come from the patchreef and lagoon floor at Y in Fig. 1. Oxygen isotopes show significant variations across the interval 140–105 kyr, while the carbon isotopes do not. $\delta^{13}\text{C}$ shows no significant variation, first because its temperature dependency is three (ref. 26) to six (ref. 27) times smaller than for $\delta^{18}\text{O}$, and second because it has greater intrinsic variability

related to local and diurnal $\delta^{13}\text{C}$ variations measured in the coastal waters²².

Concerning the $\delta^{18}\text{O}$ variations, two factors are important for organisms in well mixed ocean waters. Temperature rise of 1 °C lowers $\delta^{18}\text{O}$ by about 0.24‰; glacio–eustatic sea-level rise of 10 m lowers $\delta^{18}\text{O}$ by ~0.1‰ due to dilution of oceanic oxygen by ^{18}O -poor ice meltwater²⁸. These two factors are decoupled in Table 1 as follows. The difference between the modern *Tridacna* $\delta^{18}\text{O}$ value (-1.54%) and that for a given reef phase is treated as if it were due to sea level being different from present, giving the $\delta^{18}\text{O}$ sea-level estimates of Table 1. Should this apparent sea level be significantly different, statistically speaking, from the sea level previously established for that reef phase then the 'unexplained' $\delta^{18}\text{O}$ residual is taken as being temperature related, for example, for reef crest VI (line 2, Table 1), $\delta^{18}\text{O} = -1.45\%$ difference from modern = -0.09% , giving an apparent sea level of -9 m. This is not significantly different from the estimated sea level of -14 m.

In this way, only reef VIIb crest shows a $\delta^{18}\text{O}$ value which is not explicable in terms of sea-level change. It is 0.41‰ higher than modern; more importantly, it is 0.51‰ higher than the value for VIIa crest, which formed at a similar sea level 5–7 m higher than present (Table 1). This $\delta^{18}\text{O}$ difference is significant at better than 0.01 level (Student's *t*-test), and the difference lies between 0.40‰ and 0.62‰ at the 95% confidence level. As the sea level cannot account for this difference, we conclude that the ocean adjacent to Huon Peninsula was 1.7–2.5 °C cooler during VIIb crest building than for VIIa crest.

Cooling during VIIb crest building follows a sea-level rise of >8 m which commenced at least 1–2 kyr previously (discussed above). This agrees qualitatively with the Antarctic surge theory, which predicts both rapid sea-level rise and significant world cooling before onset of the northern glaciation. Note that the Milankovitch factor cannot explain this cooling, as radiation 120 kyr ago was similar to the present (Fig. 2c), and in any case our *Tridacna* samples measure mean annual conditions, which do not vary significantly in Milankovitch terms.

We stress that our samples from reef VIIb formation, which indicate this cooling during the sea-level rise at 120 kyr, come from the seaward side of barrier reefs which were distant from river mouths and fully circulated by open sea. This 2 °C cooling poses a quantitative problem, if it reflects the arrival in the New Guinea region of cold water originating from an Antarctic surge. If all the heat for melting the floating ice came from the entire oceans (latent + sensible heat), net cooling would be only 0.3 °C. Either there is a more direct transfer via some component of the oceanic circulation, or global cooling arising from the extended ice shelf albedo is implicated. We note that the cooling effect seems largely to have disappeared in the tropical New Guinea

Table 1 Carbon and oxygen isotope data, sea levels and ages for New Guinea reefs of last interglacial episode

Reef phase*	Location†	Age estimate‡ (kyr)	Sea level (m)	No. of specimens	Determinations	$\delta^{13}\text{C}$ (PDB ‰)	$\delta^{18}\text{O}$ (PDB ‰)	$\delta^{18}\text{O}$ sea level (m)§	$\delta^{18}\text{O}$ residual (‰)	Temperature residual (°C)
Modern	S, G	0	0	7	23	2.25 ± 0.26	-1.54 ± 0.19	—	—	—
Reef VI crest	S, G	105	-14	2	8	2.73 ± 0.11	-1.45 ± 0.07	-9	NS	0
transgn	G	110	-40	1	3	2.76 ± 0.07	-1.00 ± 0.13	-54	NS	0
Reef VIIb regn	S, G	11	-15	7	16	2.55 ± 0.25	-1.47 ± 0.14	-7	NS	0
crest	S, G	120	8	7	20	2.36 ± 0.28	-1.13 ± 0.09	-41	0.49	-2.0
Reef VIIa crest	S	133	-5	8	16	2.47 ± 0.11	-1.64 ± 0.11	10	NS	0
transgn	Z	140	-40	1	3	2.32 ± 0.05	-1.24 ± 0.14	-30	NS	0
transgn	Z	144	-60	1	4	2.66 ± 0.09	-0.80 ± 0.16	-74	NS	0

* Reefs as in Fig. 1: transgn, transgressive shallow water facies; regn, regressive facies.

† Locations according to profiles in ref. 11: S, Sialum; G, Gitua; Z, Sazum.

‡ Age estimates and sea levels according to refs.

§ $\delta^{18}\text{O}$ sea level = $(\delta^{18}\text{O}_{\text{modern}} - \delta^{18}\text{O}_{\text{sample}}) \times 10$ m.

|| $\delta^{18}\text{O}$ residual = (estimated sea level - $\delta^{18}\text{O}$ sea level) $\times 0.1\%$.

¶ Temperature residual = $(\delta^{18}\text{O}$ residual/0.24) °C. NS, not statistically significant.

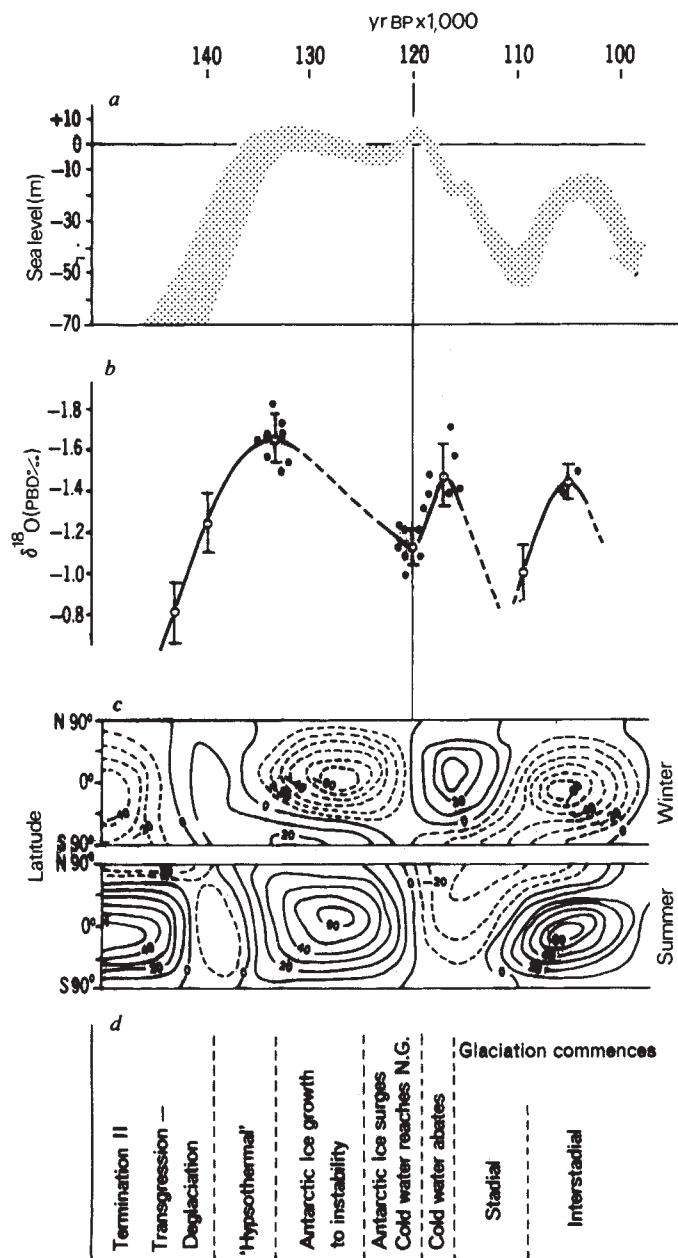


Fig. 2 Sea levels, $\delta^{18}\text{O}$, and insolation changes, 150–100 kyr BP. a, Sea levels^{10,11}; b, $\delta^{18}\text{O}$ from *Tridacna gigas*, New Guinea reefs. ●, Specimen values; ○ are group means for each stratigraphic unit, bars are group 1 σ values. c, Deviations of solar radiation from present values in Langley's per day³⁰. d, Interpretation.

about 3,000 yr after the VIIb peak, that is, when the VIIb patch reefs formed (point Y in Fig. 1), as temperatures similar to present are indicated (Table 1). (Although this result is from samples which grew within, as against outside, a lagoon, we believe lagoon ponding was not involved as the geometries are similar to modern, fully flushed lagoons at Huon Peninsula.) Hence, irrespective of the mechanism which produced the 2 °C cooling during the VIIb sea-level rise, it seems that its manifestation waned in the tropics while the northern glaciation was initiated. We do not know whether the surging ice mass was more likely to have been East Antarctica (Wilson hypothesis^{3,4}) or West Antarctica (Mercer hypothesis^{5,6}).

Note also that the Milankovitch factor predicts strong warm-summer-cool-winter contrast during the 125–130 kyr interval, a condition normally taken as inimical to northern continental ice growth. Such a radiation pattern may favour enhanced summer snowfall in Antarctica, leading to icesheet building towards

instability. This might explain the minor sea-level decline from the VIIa peak before the VIIb rise. Figure 2d summarises the sequence of events, and we conclude that an Antarctic ice surge seems likely to have initiated the last glaciation.

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Isostatic equilibrium grounding line between the West Antarctic inland ice sheet and the Ross Ice Shelf

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The Ross Ice Shelf is widely believed to be a Holocene feature, that is, during the late Pleistocene the inland (grounded) ice sheet extended out nearly to the continental shelf break in the Ross Sea, and retreated to its present position between 5,000 and 10,000 yr BP. If so, and if the grounded ice sheet remained in that position for at least 10,000 yr, then a sea floor uplift of the order of 100 m is still to be expected in the grid western part of the Ross Ice Shelf. Even for a smaller and more ephemeral extension of the grounded ice, the uplift would still be several tens of metres. Recently reported measurements indicate that there are extensive areas near the present grounding line where the water layer beneath the ice shelf is thin enough so that uplift would lead to a grounding line advance of at least 100 km. As the sea floor depth generally increases towards the grid east, however, the maximum extent would not be past the middle of the present Ross Ice Shelf.

Seismic soundings of submarine topography and radar soundings of ice thicknesses were made at sites on a 55 km grid covering the Ross Ice Shelf, Antarctica, during the Ross Ice Shelf Geophysical and Glaciological Survey (RIGGS)^{1–5}. The submarine topography, characterised by a general deepening to

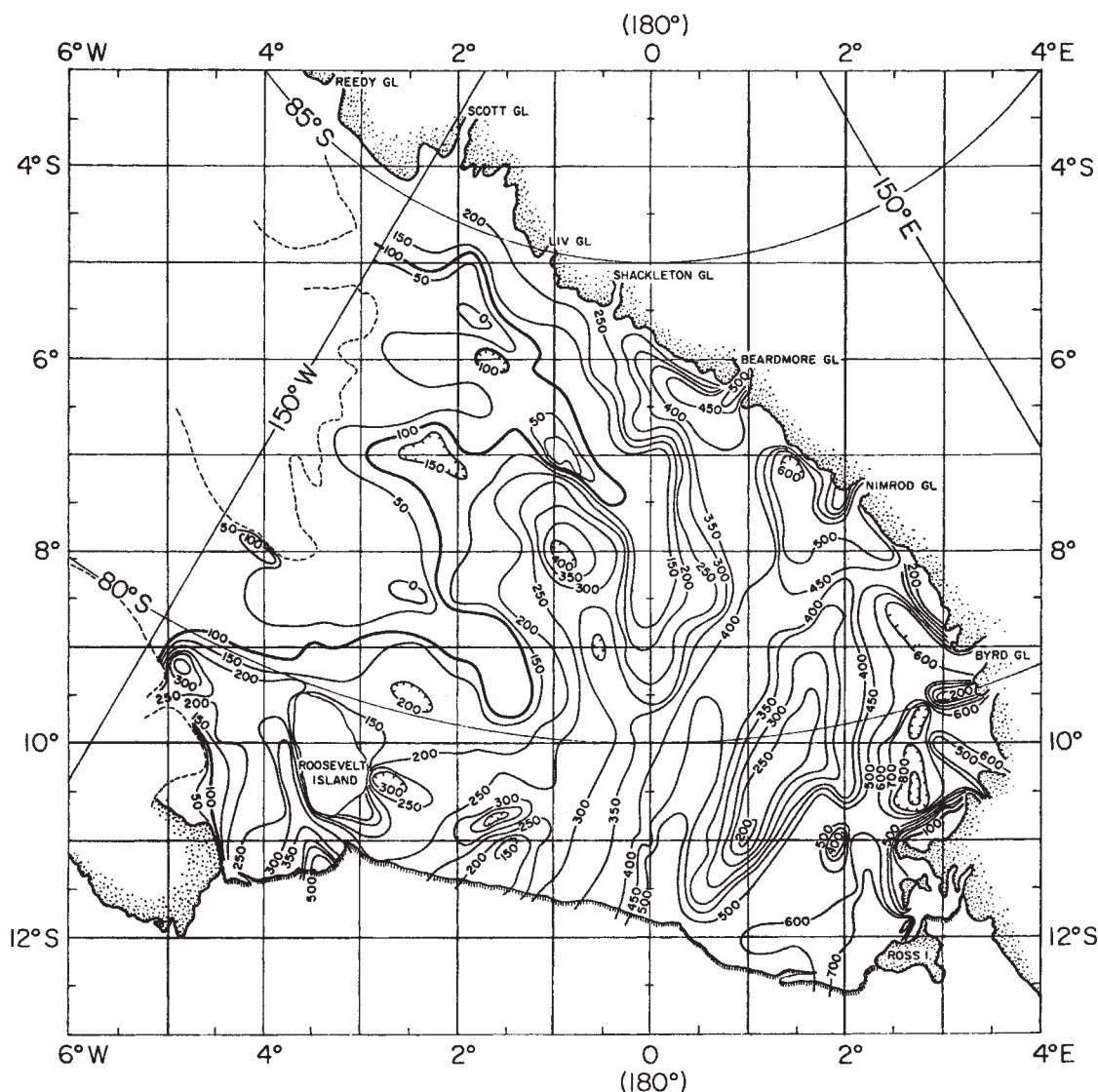


Fig. 1 Water depth beneath the Ross Ice Shelf. Standard grid coordinates are shown; grid north is towards Greenwich, and grid meridians are parallel to 180° W longitude. The present grounding line between the Ross Ice Shelf and West Antarctica is shown by the dashed line which is discontinuous in areas where ice streams enter the ice shelf. The contour interval is 50 m for depths from 0 to 500 m, and 100 m for greater depths.

the grid eastwards on which is superimposed a curving ridge-trough system, is reflected in a contour map of the 'water depth' (defined as the thickness of the water layer beneath the ice shelf) (Fig. 1). The position of the primary grounding line between the Ross Ice Shelf and West Antarctica was determined from airborne radar sounding measurements of ice surface elevations and strength and polarisation of bottom reflections⁶; the latter indicate whether water or rock is present directly below the ice. Seismic, radar and altimeter measurements made on the surface¹⁻³ were used for control. The surface topography shows the grounding line to be deeply indented beneath the West Antarctic ice streams, although scattering of the radar signals by heavy crevassing prevents accurate determination of its position. The ice shelf is also grounded at Roosevelt Island (grid position 10.0° S, 3° W), Cray Ice Rise (7.0° S, 1.0° W), near the Steershead Crevasses (8.5° S, 2.5° W), and possibly at RIGGS station F7 (5.5° S, 1.8° W) (see Fig. 3). Beneath much of the grid western part of the ice shelf, the water depth is <100 m, and in some places it is only a few tens of metres.

Stuiver *et al.*⁷ have reconstructed the Antarctic Ice Sheet at the 18,000 yr BP glacial maximum. Their reconstruction indicates that West Antarctic ice was then ~2,500 m thick near the present grounding line, and that the margin coincided with a line of sills parallel to the edge of the Ross Sea continental shelf. Two-by-two-degree mean isostatic gravity values at RIGGS stations on the Ross Ice Shelf⁸, which are as much as 15 mGal

negative compared with a fourth-order satellite gravity field⁹, strongly suggest that glacio-isostatic depression of the grid western part of the present Ross Ice Shelf area still remains. Recent evidence from Ross Sea cores also suggests that grounded ice reached the continental shelf margin during the late Pleistocene¹⁰. Thomas and Bentley¹¹ calculated that if such a Ross Ice Sheet existed 18,000 yr ago, then it probably retreated rapidly to its present position between 13,000 and 7,000 yr BP. Geological data in the McMurdo Sound and dry valley areas yield minimum ages of ice retreat ranging from 3,000 to 10,000 yr BP (ref. 12) and have further been interpreted as showing that the grounding of the ice sheet occurred more than 47,000 yr ago¹³.

Although some recent analyses of marine and glacial geological evidence indicate that only minor expansions of the grounded ice occurred during the Quaternary¹⁴⁻¹⁶, we believe that the preponderance of evidence favours the existence of an extended grounded ice sheet of some form. The following analysis is based on the initial working hypothesis that a grounded 'Ross Ice Sheet' existed long enough to become isostatically compensated (~10,000 yr), and that it was removed rapidly between 5,000 and 10,000 yr BP. In that case, isostatic uplift of the loaded area should still be occurring. The grounding line between the West Antarctic Ice Sheet and the Ross Ice Shelf will advance as the sea floor rises. We have estimated the uplift remaining in the grid western portion of the Ross Ice Shelf,

assuming a former ice thickness of 2,500 m (ref. 7). Following Bennett¹⁷, we take unloading to have been instantaneous, and assume a simple exponential model of uplift:

$$Z = Z_0 \exp[-(t - t_0)/t_r] \quad (1)$$

where Z is the amount of uplift remaining, Z_0 is the original isostatic depression due to the ice load, t_0 is the time of load removal and t_r is the time constant of isostatic rebound. Z_0 can be estimated from the former ice thickness, T , and the estimated average depth of the present sea floor, $\bar{D}$, by

$$Z_0 = \frac{\rho_i}{\rho_m} T - \frac{\rho_w}{\rho_m} (\bar{D} - Z) \quad (2)$$

where ρ_i , ρ_w and ρ_m are densities of ice, sea water and mantle rock, respectively. Substitution of equation (2) into equation (1) yields an expression for the remaining uplift:

$$Z_p = \frac{\left[\frac{\rho_i}{\rho_m} T - \frac{\rho_w}{\rho_m} \bar{D} \right] \exp[-(t_p - t_0)/t_r]}{1 - \frac{\rho_w}{\rho_m} \exp[-(t_p - t_0)/t_r]} \quad (3)$$

wherein Z_p and t_p are the present remaining uplift and the present time.

The average water depth at RIGGS stations, $\bar{D}$, is ~650 m. The relaxation time constant, t_r , was estimated for an area the size of the Ross Ice Shelf from values measured in the Northern Hemisphere and predicted by model studies (Fig. 2, adapted from Cathles¹⁸). The linear dimension of the Ross Ice Shelf is of the order of 1,000 km, so t_r was chosen to be ~4,400 yr, the value used by Cathles for central Fennoscandia. Note that t_r is relatively independent of both the linear dimension of the ice load and the crustal rigidity for ice loads of this size. Densities used in equation (3) were $\rho_i = 0.91 \text{ Mg m}^{-3}$, $\rho_w = 1.03 \text{ Mg m}^{-3}$, and $\rho_m = 3.33 \text{ Mg m}^{-3}$.

The results of the calculation (Table 1) show a remaining uplift ranging from 50 to 170 m. This estimate agrees well with that from the mean isostatic gravity anomalies which suggest as much as 150 m residual depression near the grounding line⁸. Because of the linearity of equation (1), these figures can easily be modified to fit other assumptions. Thus, for example, if the initial depression were only half as large as has been assumed, the uplift remaining would still be at least 50 m if the time since removal is less than 7,000 yr. That initial depression, in turn, could correspond to a fully compensated grounded ice sheet whose margin extended only about as far as a line extending from Edward VII Peninsula to Byrd Glacier, to a fully extended ice sheet which was in position for only ~3,000 yr, or to a 'low profile' extended ice sheet that might be compatible with the shape of internal reflecting horizons found in the West Antarctic interior²⁷. Thus 50 m is a reasonable approximation to an uplift to be expected even assuming a smaller or more ephemeral ice sheet.

Table 1 Uplift remaining since ice removal

Time since ice removal (yr)	Remaining uplift (m)
5,000	170
6,000	135
7,000	105
8,000	80
9,000	65
10,000	50

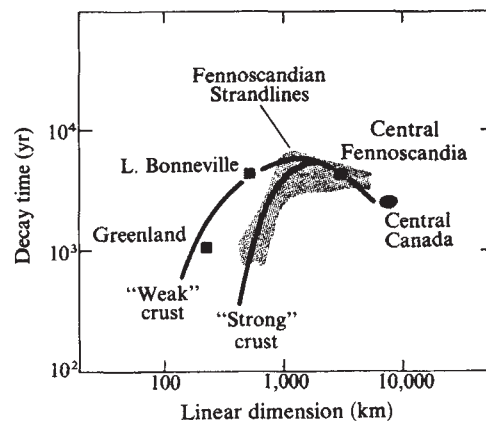


Fig. 2 Decay time constant for isostatic uplift versus the linear dimension of areas formerly covered by ice sheets (Canada, Greenland, Fennoscandia) or water (Lake Bonneville). Adapted from Cathles¹⁸.

We have, therefore, estimated a maximum and minimum predicted advance of the grounding line based on a remaining uplift of 50–170 m and the assumption that the ice shelf thickness remains constant at the grounding line (Fig. 3). The maximum predicted grounding line extends grid northeastwards across the ice shelf from the seaward end of Roosevelt Island, and is deeply indented by extensions of the present ice streams. The minimum predicted grounding line advance would result from an advance of the central part of the present grounding line, with additional minor advances occurring near Edward VII Peninsula, Roosevelt Island and Crary Ice Rise.

The present rate of uplift is, from equation (1):

$$\left. \frac{dZ}{dt} \right|_{t=t_p} = -\frac{Z_p}{t_r} \quad (4)$$

for $Z_p = 110$ m, the uplift rate is 25 mm yr^{-1} . Division by the water depth gradient, which varies from 10^{-4} to 10^{-3} near the present grounding line, yields a rate of advance of the grounding line of $25\text{--}250 \text{ m yr}^{-1}$. At this rate, several thousand years would be required for the grounding line to advance to its predicted maximum position. As uplift occurs, however, points seaward of the primary grounding line will be pinned on high points of the sea bed, forming new ice rises. Compressive strains upstream from these pinning points will greatly accelerate the rate of grounding line advance. We believe this is now occurring upstream of Crary Ice Rise (grid 7.0° S , 1.0° W)¹⁹, and also near the Steershead Crevasses (grid 8.5° S , 2.5° W).

The possibility that sedimentation may have been an important factor during isostatic adjustment can be dismissed on the basis of ocean floor cores taken at station J9 (grid 7.5° S , 1.5° W). Little or no Pleistocene or Recent sediment is present at that location²⁰, whereas a sedimentation rate of the order of 55 mm yr^{-1} would be required to produce a present state of isostatic equilibrium¹⁷. Furthermore, even if the basal layers of the ice shelf contain as much as 10% morainal material, the latter sedimentation rate would imply a bottom melt rate of $\sim 0.5 \text{ m yr}^{-1}$, whereas temperature and resistivity measurements indicate a near-zero melt-freeze rate at J9 (ref. 21). Also, preliminary observations on ice cores from the lowest layers, obtained at J9 in December 1978, suggest an average bottom freezing rate of $\sim 0.03 \text{ m yr}^{-1}$ (J. W. Clough and I. A. Zotikov, personal communication).

Peltier²² has pointed out that the longest wavelength components of the depression may 'feel' the Earth's core, and decay non-exponentially. On any realistic Earth model, however, that applies only to wavelengths of many thousands of

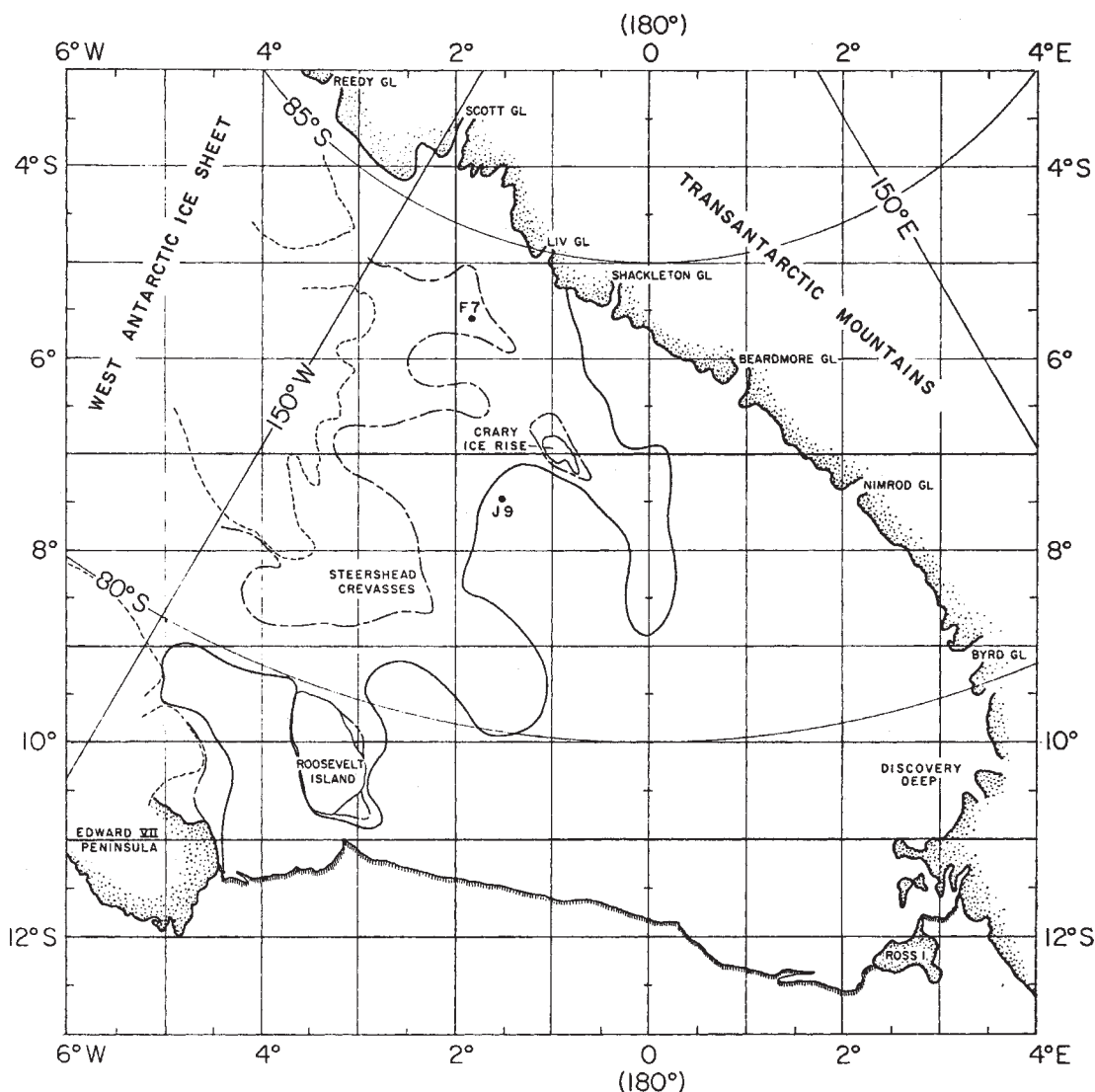


Fig. 3 Minimum (dashed line) and maximum (solid line) predicted seaward advance of grounding line, based on 50–170 m of additional isostatic uplift.

kilometres—much larger than the Ross Ice Shelf. Using Peltier's²² Green functions in the Farrell–Clark²³ model and the Stuiver *et al.*⁷ Antarctic reconstruction, Lingle and Clark²⁴ have computed a relative sea-level curve for 2,000 yr BP to the present near the centre of the Ross Ice Shelf. As their curve indicates a rate of emergence ($\sim 11 \text{ mm yr}^{-1}$) that is nearly constant rather than exponentially decreasing with time, it is impossible to use their method at the current stage of development to estimate the remaining uplift. Furthermore, as exponential decay describes well the uplift data from central Canada^{25,26}, it seems to be a reasonable assumption for uplift of the sea floor beneath the Ross Ice Shelf.

The model presented here does not, of course, consider the possibility of non-equilibrium of the West Antarctic ice sheet. Growth or shrinkage of that ice sheet due to climatic or inherent dynamic causes could produce grounding line movement which is large compared with that from isostatic uplift. However, if the West Antarctic inland ice sheet is approximately in steady state, an advance into the Ross Ice Shelf is still to be expected. The corresponding lowering of world sea level would be of the order of 0.5 m.

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Widespread episodes of stream erosion during the Holocene and their climatic cause

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Quaternary fill-terrace sediments along a 6-km reach of the Pomme de Terre River in south-central Missouri (38°06' N, 93°26' W) have yielded abundant vertebrate fossils, pollen, and archaeological remains¹. Numerous radiocarbon dates have also been obtained. When these are combined with stratigraphic results (described by Haynes and myself), an 'alluvial chronology' of floodplain depositional history can be constructed. The Holocene portion of this chronology is summarised here and compared with other dated alluvial and proxy-climatic sequences from the northern mid-latitudes. From such a comparison, considerable insight may be gained into the operation of synoptic climatological controls over fluvial sedimentation and erosion.

The state of our current knowledge on the floodplain history of the Pomme de Terre River is given in Fig. 1 which shows a plot, on an x-y coordinate system, of the sample elevations above local river level of 21 radiocarbon dates, and also the measured elevations of the tops and bottoms of each mapped stratigraphic unit. Full details regarding local stratigraphy exposed along this valley by backhoe-trenching are given elsewhere^{2,3}.

Periods of slow or fast floodplain aggradation, stability and erosion during the past 11,000 yr are immediately recognisable on Fig. 1. One facet of river history made especially evident is that conditions of 'grade' or stability have prevailed for only limited periods of time during the Holocene. Instead: (1) a major aggradation occurred between 10,500 and 8,100 yr BP, coevally with loess deposition on local hilltops²; (2) following a brief erosional event between 8,100 and 7,500 yr BP, the river rapidly readjusted its grade; (3) floodplain stability prevailed from 7,500 to ~5,000 yr BP; (4) by 4,600 yr BP a major change in long-term stream regime had occurred; and (5) since this time, rapid aggradation has alternated with closely-spaced intervals of erosion. This last period, characterised by lack of long-term stability, continues to the present.

The complexity of this Holocene alluvial sequence is of special significance to workers who map river terraces on the basis of their relative elevations above river level. The lowest well defined terrace by the Pomme de Terre River is the 'T1' level, about 4 m above the modern floodplain (T0). However, a pronounced break in slope between this terrace level and T0 is visible at only a few locations in the valley, where fortuitous erosion has removed intervening fills at intermediate levels between T1b2 and T0 (Fig. 1). Given this situation, some alluvial terraces along other rivers may well signify primarily that portions of the local depositional record are missing.

Comparison of the Pomme de Terre's floodplain history with regional long-term climatic trends, as evidenced by pollen-derived vegetational history, suggests a correlation between the two. The 7,500–5,000 yr BP period of floodplain stability along this river occurred during the 8,700–5,000 yr BP 'Prairie Maximum' in Missouri⁴. This former plant geography is believed to indicate a climate characterised by less effective precipitation, because the Prairie/Deciduous Hardwood Forest boundary was located far to the east of its modern location. Following the return of more moist regional climates ~5,000 yr BP, long-term stream regime changed. Closely spaced episodes of stream erosion and deposition began, and have continued up to the present.

What is the controlling factor causing long-term floodplain stability or instability along this stream? The timing of mid-Holocene stability along the Pomme de Terre is similar to the time of stability concluded by Haynes for many western US streams: 7,100–5,700 yr BP (ref. 5). This tends to dismiss Prairie Maximum grassland vegetation itself as the primary cause of floodplain stability: no such vegetation has been inferred for the mid-Holocene in the now semi-arid western US stream basins. A better hypothesis is that stability in both Missouri and the western US was the direct result of greatly increased recurrence intervals for moderately sized floods, due to pronounced zonal upper atmospheric circulation patterns at this time^{6,7}.

Comparing the Pomme de Terre sequence with others in the northern mid-latitudes tests a corollary to this hypothesis: that climatic periods of very high meridionality in upper atmospheric circulation should be accompanied by periods of instability along streams⁷. Horizontal bars extending temporally to the oldest and youngest date from each Pomme de Terre depositional unit are shown in Fig. 1. Gaps between these bars are the time intervals bracketing fluvial erosion at this site. Following this same method, two other published alluvial sequences were plotted adjacent to the Missouri chronology on Fig. 2. Haynes⁵ produced the western US chronology by compiling radiocarbon dates from lithostratigraphic units mapped over a large portion of the western US. He described the considerable dendrochronological and archaeological evidence in the western US for an erosional interval some time between 750 and 450 yr BP, although this event was not shown by the few radiocarbon dates then available for this period. Recent ¹⁴C dating of alluvium in northern Arizona confirms regional erosion ~450 yr BP (W. Robinson, personal communication) and this event is therefore also indicated on Fig. 2 for comparison. The central European chronology is my compilation of results presented by others^{8–11}. It is based partly on dates obtained by dendrochronological analysis of fossil tree stumps and logs found in alluvial deposits. Because such dates are in calendar and not radiocarbon years BP, every such tree-ring-based date from these studies was first converted into radiocarbon years BP using a standard calibration curve¹² before plotting on Fig. 2.

The three alluvial chronologies thus compared show four coincident or overlapping time intervals bracketing fluvial erosion (black bars on Fig. 2). Stream erosion at 5,000–4,900, 2,900–2,500, 1,600–1,500 and 400–500 radiocarbon yr BP is compatible with all three alluvial sequences.

Other recent alluvial chronological work supports the existence of very widespread Holocene erosional episodes at these times. In southwestern Wisconsin, Knox and his students have demonstrated regional stream erosion between 6,000 and 8,000 yr BP, before 4,400 yr BP, before 3,100 yr BP and

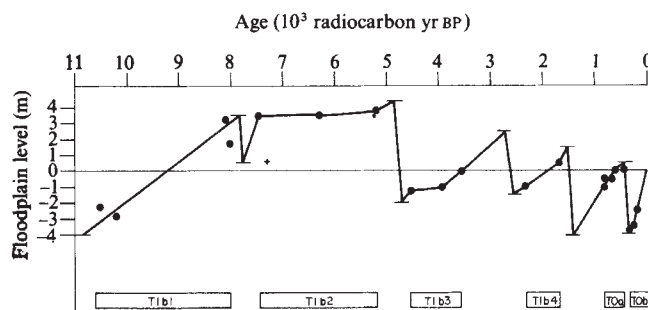


Fig. 1 Holocene level history of the Pomme de Terre River, southern Missouri, US. ●, Alluvial radiocarbon dates; + shows one of these dates that has poor reliability due to a large laboratory standard deviation. Short horizontal lines are undated stratigraphic tops or bottoms of each alluvial unit. Elevations are in metres above or below the modern floodplain, set at zero. Laboratory numbers of dates shown on this diagram are: ISGS-35, 48; SMU-429, 430, 431, 485, 500; UCR-819, 820, 823, 824; M-1900, 2281, 2333; GAK-1170, 1172; TX-1411, 1454, 1466; and A-867.

between 1,800 and 1,225 yr BP (ref. 13). At the Koster site in southern Illinois, Butzer infers stream erosion at 5,000, 2,000 and 1,200 yr BP (ref. 14), although the latter two episodes are "fixed in relation to the regional archeological sequence" (personal communication). In Austria, along the Danube River, Fink's dates and cross-sections¹⁵ support erosion between 8,500 and 7,000 yr BP, shortly before 4,200 yr BP, before 2,390 yr BP, shortly before 1,700 yr BP and between 560 and 400 yr BP. Finally, in Poland, Jersak dates an erosional unconformity in alluvial sediments between 8,620 and 6,160 yr BP¹⁶, and Lindner presents a cross-section showing the basal portion of an alluvial fill dated at 1,850 and 2,060 yr BP (ref. 17).

Together, this is convincing evidence that sporadic episodes of widespread stream erosion have occurred during the past 5,000 yrs in the northern mid-latitudes. This result does not contradict dis-synchronicity of alluvial sequences at other times. Thus, stream erosion between 8,500 and 6,000 yr BP occurred at most localities mentioned above, but the data so far available are compatible with either: two closely spaced erosional intervals centering on 7,700 and 6,500 yr BP, and perhaps felt only locally, or one time-transgressive event between 8,000 and 6,500 yr BP, beginning earlier in eastern Europe and central North America, and later in central Europe and western North America.

The cause of the widespread stream erosion at the times shown in Fig. 2 is suggested by the timing of the Northern Hemisphere 'little ice ages'. These chronologies of mountain glacier advances have been assembled by Quaternary geologists working largely independently. However, their work shown in Fig. 2 demonstrates that Holocene glacial activity has been clustered around certain time intervals, even when the most divergent record (no. 6 in Fig. 2) is included. Widespread fluvial erosion seems to occur approximately at the beginning of little ice age climatic phases.

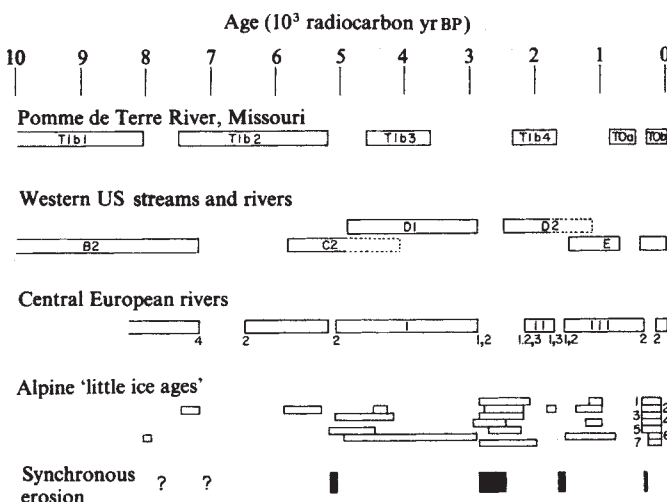


Fig. 2 Holocene alluvial and glacial chronologies compared. Key numbers 1–4 for 'Central European Rivers' refer to refs 8–11, respectively. Key numbers for 'Alpine Little Ice Ages' indicate the following references: 1, Alaska, ref. 21; 2, Swedish Lapland, ref. 21; 3, British Columbia and northern Washington, ref. 22; 4, Norway, ref. 23; 5, glaciations common to the eastern and western Alps, ref. 24; 6, Colorado Rocky Mountains, ref. 25; 7, Mexican volcanoes, ref. 26.

Other proxy palaeoclimatic indicators support climatic change occurring at these times, and help to clarify the nature of these changes. According to the California tree-ring results of LaMarche from both upper and lower treeline¹⁸, the times of widespread glacial advances and stream erosion were characterised by rapidly decreasing land temperatures and increasing precipitation. Unfortunately, there are no such very long tree-

ring climate series from the central US or Europe, and most pollen data from the central US are not sensitive enough to record short-lived climatic changes. However, "reconstructions of late-Holocene positions of treeline in Keewatin and Mackenzie, NWT, Canada, indicate that treeline was located south of its present position at about 2,900, 1,800 and 800 radiocarbon yr BP" and this is compatible with "increases in the strength of the meridional component of the large scale upper atmospheric circulation" at these times⁷. Moreover, Frenzel concludes that a variety of palaeoclimatic indicators from southwestern Europe also support greater meridionality at 5,200–4,400, 3,500–3,000, 1,700–1,200, and 800–600 yr BP, with shorter cooler and/or wetter phases ~2,700–2,500 yr BP, and within the past 600 yr⁶. Certainly it is hazardous to attempt detailed climatic comparisons over large areas on the basis of diverse palaeoclimatic indicators. Yet certain large scale changes have apparently left relatively unambiguous traces. If climatic change was the ultimate cause for recurring episodes of stream erosion during the past 5,000 yr, then the assembled palaeoclimatic evidence favours intensified meridionality in upper atmospheric circulation as the direct cause. This hypothesis was first advanced by Knox⁷, on the basis of a statistical analysis of alluvial radiocarbon dates.

Because river channels are formed by floods with a recurrence interval of between 1 and 2 yr¹⁹, channel widening or vertical channel incision, therefore, occurs whenever floods of such recurrence intervals increase in size. During modern times, significant increases in meridional upper atmospheric circulation have been shown to result in such flooding over large areas²⁰. Therefore, widespread episodes of Holocene stream erosion are to be expected if climatic regimes characterised by highly meridional circulation occurred in the past. Proxy palaeoclimatic records suggest that such regimes did occur, and at the correct times. Positive glacial mass balances in several mountain ranges resulted, even as increased flooding and discharge occurred along lowland streams. In contrast, one long interval of predominantly zonal flow was characterised by floodplain stability.

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Age of volcanism and rifting in southwestern Ethiopia

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It has been suggested that volcanism in the Ethiopian region of the Afro-Arabian Rift System has migrated with time, both laterally towards the present axial zone¹⁻³ and longitudinally southwards from the Red Sea^{4,5}. Field data and K-Ar isotopic ages from southwestern Ethiopia, summarised below, indicate that volcanism in this area began earlier than previously suspected, and that Quaternary volcanism was not restricted to the axial zone. That major rifting in this region did not happen until the late Miocene^{6,7} is corroborated.

The Afro-Arabian Rift System in southwestern Ethiopia is particularly wide and complex⁸, embracing from east to west the southern termination of the Main Ethiopian Rift (Ganjuli graben^{9,10}), the Stefanie Rift, and three northward-terminating branches (Usno, Omo and Kibish) of the Turkana (Lake Rudolf) Rift. Rugged terrain between the Ganjuli graben and the western edge of the Ethiopian Highland, 450 km north-west, exposes crystalline basement overlain unconformably by various Tertiary and Quaternary volcanic rocks^{11,12} (Fig. 1).

The Tertiary volcanic rocks are broadly divisible into two categories, based on whether erupted before rifting or not. Among those in the pre-rift category, some relative age distinctions can be made based on local unconformities within flow successions or on the nature of the unconformity on crystalline basement. The oldest suite, referred to as the main sequence, overlies crystalline basement with a characteristic, thin, basal unit of red, indurated, residual grit. Continuity of this uniform grit, not faulted or eroded before covering by lava, coupled with lack of both organic remains and evidence of fluvial origin, suggest that most of this region was a featureless, dry peneplain, probably at low elevation, at the inception of Tertiary volcanism. Younger flows, generally conformable on and thus difficult to separate from the main sequence in the central north-east part of the area, overstep the main sequence on to basement to the west, south-west and south, and all lack a basal, red grit. Phonolite flows and small intrusions respectively overlie and cut all earlier flows and comprise the youngest pre-rift igneous rocks.

In the post-rift category, flood basalt sheets lie with marked unconformity on tilted pre-rift flows and are channelled by rift-related topography. Quaternary volcanism not only occurred locally within the Stefanie and Turkana Rifts but also on adjacent highland blocks, notably in the north-west where it built a huge shield astride the Tertiary unconformity, far removed from the axial rift zone.

Conventional K-Ar isotopic ages were obtained by analytical procedures described by Briden *et al.*¹³ for basalt samples collected throughout the area from the base of successions that lie on crystalline rocks. Ages for rhyolite within the main sequence and for two phonolite intrusions were also determined. Age data are given in Table 1; sample locations are plotted in Fig. 1. Of 12 samples from the base of the main sequence, five cluster at ~40 Myr, three from near the Sudan border are distinctly older at ~48 Myr, one (C774) is anomalously old, and three are significantly younger. Of the latter, K095 is judged anomalous because it lies several hundred metres below rhyolite (A515) dated at 32.7 ± 1.3 Myr. Two periods of extensive younger basalt effusion are documented by

clusters of ages at ~32 Myr (Gok and Makonnen basalts in the north-west, Fejej basalt east of Lake Turkana) and ~20 Myr (Teltele basalt in the south-east and Surma basalt near the Sudan border). Ages of C775, 23.1 Myr, and B425, 54.7 Myr, are unexplained anomalies at unconformities where other age data are consistent. Phonolite in the Stefanie Rift is dated at ~13 Myr. Basalt (4.2 Myr) in outliers east of Lake Turkana is much younger than the Fejej basalt which it oversteps.

The age data accord well with published ages from the same region. Early main sequence flows are of the age suggested for the pre-Oligocene Ashangi basalts of central Ethiopia^{2,10}. Makonnen, Gok and Fejej basalts are similar in age to the Aiba basalts of central Ethiopia^{1,2,15,16} and to early basalts in northern Kenya¹⁷. 35–37 Myr ages obtained from low in the main sequence near the south end of the Main Ethiopian Rift are midway between these two groups, and 31 Myr old flows occur higher in the same succession without an unconformity between them^{9,10}. The much younger Teltele and Surma basalts have probable age equivalents in flows overlying Fejej basalt east of Lake Turkana¹⁸, and in the middle part of the pre-rift succession in the lower Omo Valley¹⁹. Phonolite igneous activity is the same age as in adjacent Kenya^{17,20}. The post-rift basalt north-east of Lake Turkana correlates closely in age with the Mursi basalt in the Omo Valley^{7,19}, and with the Harr basalt east of Lake Turkana (R. T. Watkins, personal communication).

Table 1 Sample data

Location, map-unit, and position in section	No. in Fig. 1	Sample no.	%K	Volume radiogenic ⁴⁰ Ar (std. cm ³ g ⁻¹ × 10 ⁻⁶)	% radiogenic ⁴⁰ Ar	Age (Myr)
Main sequence						
Sudan-Ethiopia border; analcime basalt flows on crystalline basement. Basal red grit not observed	1	A484	0.599	1.0989	19.5	46.6 ± 2.0
	2	B608	0.437	0.8349	32.1	48.5 ± 1.9
	3	I045	0.427	0.8312	41.5	49.4 ± 1.9
Basal flood basalt flows, unconformable on crystalline basement with basal grit (a) observed at sample site, (b) present along same segment of unconformity, (c) not observed at or near sample site, (d) not present, flow unconformable on >10 m mafic tuff	4 (a)	A251	0.732	1.1616	65.3	40.4 ± 2.0
	5 (a)	A313	1.046	1.7573	58.4	42.7 ± 2.0
	6 (a)	A627	0.947	1.3114	61.7	35.2 ± 1.4
	7 (d)	B658	0.616	0.9204	29.3	38.0 ± 1.6
	8 (c)	B659	1.250	1.9769	60.9	40.1 ± 1.2
				1.9630	60.6	
				4.6406	49.9	95 ± 4*
				4.6442	46.9	
	9 (b)	C774	1.220	0.6812	44.2	33.9 ± 1.3
	10 (b)	D191	0.512	1.5690	40.8	39.0 ± 1.5
	11 (c)	D409	1.024	1.3258	64.9	
	12 (b)	K095	1.158	1.4450	63.0	30.5 ± 1.0*
Sanidine vitrophyre flow overlying several hundred metres of flood basalt with basal red grit	13	A515†	3.086	3.8720	59.6	32.7 ± 1.3
				4.0333	47.3	
Younger pre-rift sequences						
Gok basalt; (e) basal flow, (f) number of flows above base not known, but less than 5; unconformable on crystalline basement or Permian sandstone	14 (f)	A718	0.963	1.0865	28.2	28.8 ± 1.1
	15 (e)	A735	0.761	1.0386	28.2	34.8 ± 1.3
	16 (f)	B799	1.397	1.6690	59.9	30.5 ± 1.2
	17 (e)	C775	0.954	0.8640	58.7	23.1 ± 1.2*
	18 (f)	D677	0.777	0.9716	22.5	31.9 ± 1.2
Makonnen basalt; (g) basal flow, (h) third flow above base; unconformable on crystalline basement	19 (g)	B683	1.255	1.5549	59.0	31.6 ± 1.2
	20 (g)	B685	0.759	0.9269	42.9	31.1 ± 1.2
	21 (h)	C695	1.068	1.3179	70.3	31.5 ± 1.0
	22 (g)	K002	0.754	0.9699	48.6	32.8 ± 1.2
Fejej basalt, basal flow on crystalline basement	23	C256	0.614	0.7897	55.0	32.8 ± 2.0
Teltele basalt, basal flows; (i) separated from crystalline basement by 6-m tuff layer	24	B125	1.918	1.5726	57.7	21.0 ± 0.8
	25 (i)	B247	0.475	0.3476	38.1	18.7 ± 0.8
	26	B334	1.079	0.8956	28.3	21.2 ± 1.0
				1.4683	31.4	
	27	B425	0.665	1.4019	32.3	54.7 ± 2.0*
Surma basalt, basal flow on crystalline basement	28	D509	1.210	0.9157	30.4	19.4 ± 0.6
				0.9148	28.4	
Stefanie Rift; pre-rift hypabyssal phonolite intrusions	29	A033	4.08	2.0639	4.9	13.0 ± 2.4
	30	A034	4.25	2.1130	13.4	12.7 ± 1.0
Post-rift sequence						
Basal basalt flow, outlier on crystalline basement north-east of Lake Turkana	31	A164	0.897	0.1394	22.2	4.2 ± 0.4
				0.1504	18.5	

$\lambda_a = 4.962 \times 10^{-10} \text{ yr}^{-1}$; $\lambda_e = 0.58 \times 10^{-10} \text{ yr}^{-1}$; $^{40}\text{K}/\text{K} = 0.01167 \text{ atom } \%$ (Steiger and Jager¹⁴).

* Ages considered anomalous.

† Determined on sanidine extract; all other determinations are whole rock.

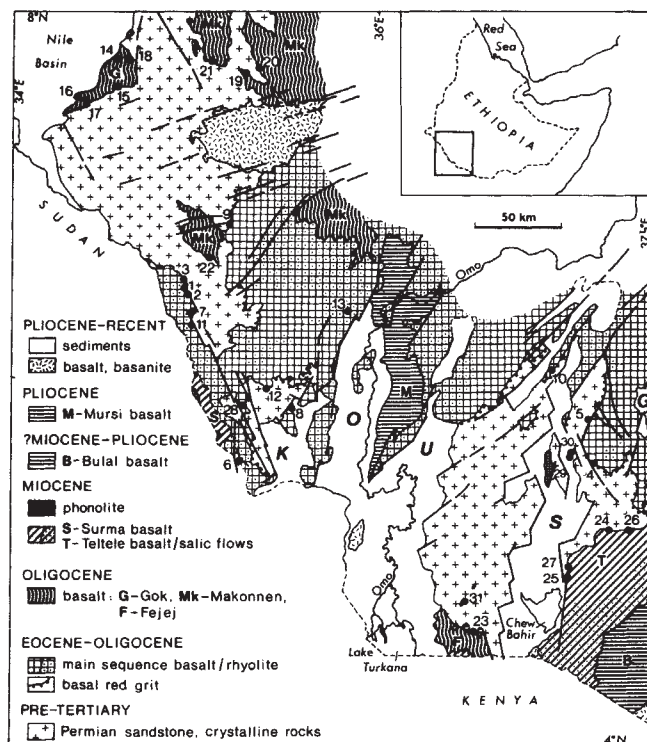


Fig. 1 Geology, southwestern Ethiopia, and location of dated samples. G, Ganjuli graben; S, Stefanie Rift; U, O and K, Usno, Omo and Kibish branches of Turkana Rift.

We conclude: (1) volcanism began as long ago as the middle Eocene and was widespread by the late Eocene in south-western Ethiopia. Early flows were deposited on a peneplain, likely at low elevation. Whether these flows were once continuous with the Ashangi basalts of central Ethiopia, or whether the two relate to separate broad centres of Eocene volcanism is not known. (2) Throughout the Oligocene, a broad arch developed, concave to the north-east, with uplift maxima in the north-west and south-east parts of the area. Oligocene and Miocene lavas covered older flows and overlapped on to basement, in part re-exposed, on top of and flanking the south and west sides of this arch. (3) Major rift structures did not form across this uplift until after Miocene (13–12 Myr) phonolite volcanism, although shallow proto-rifts may have existed somewhat earlier²¹. Rift topography was fully developed by the early Pliocene (5–4.5 Myr). (4) Correlation of Gok and Makonnen basalts, whose base elevations differ by 1.5 km, implies major faulting at the western edge of the Ethiopian Highland. (5) Quaternary volcanism was not restricted to the axial rift zone, but occurrences outside this zone are spatially related to new developing rifts or fracture zones. These discoveries in southwestern Ethiopia require that the pattern of migration of volcanism with time in the Ethiopian region of the Afro-Arabian Rift System be re-assessed.

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Free calcium in *Xenopus* embryos measured with ion-selective microelectrodes

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A regulatory role for intracellular free calcium has been suggested in cell division¹ and intercellular communication via gap junctions^{2,3}. Previous measurements of cytoplasmic free Ca^{2+} during cell division and uncoupling have been made with the Ca^{2+} -sensitive photoprotein aequorin^{4,5}. We now report experiments using Ca^{2+} -sensitive microelectrodes to monitor free Ca^{2+} in the cells and intercellular fluid of early embryos of the amphibian *Xenopus laevis*. In addition to measuring basal levels, we have looked for changes in free Ca^{2+} during cell division and during electrical uncoupling of the normally coupled embryonic cells, induced by acidification of the intracellular medium^{5–7}.

Ca^{2+} -selective microelectrodes, tip size 1–2 μm , were made with a neutral ligand sensor based on that originally described by Oehme *et al.*⁸. The micropipettes were silicized with tributylchlorosilane vapour at 200 °C. The sensor contained 2.4% tetraphenylphosphonium bis(1,3-diethyl-2-thio-barbiturate)trimethineoxonol in place of the 1% sodium tetraphenylborate commonly used in this type of electrode. Details of the fabrication, calibration and properties of these electrodes will be published elsewhere (R.Y.T. and T.J.R., in preparation). In the presence of 0.1 M KCl, the electrodes showed ideal (Nernstian) responses between pCa 3 and pCa 6 (1 mM to 1 μM free Ca^{2+}), 12–20 mV between pCa 6 and 7, and 4–8 mV between pCa 7 and 8. Interference from H^+ and Mg^{2+} was negligible. At pCa 7 and 6, alterations in pH between 7.8 and 6.5 produced no detectable electrode response. Elevation of free Mg^{2+} from 0 to 5 mM or of Na^+ from 0 to 20 mM had less effect on the electrode potential than raising free Ca^{2+} from 0 to 0.1 μM . The free Na^+ in *Xenopus* embryonic cells is probably no more than 20 mM (ref. 9). High concentrations of CO_2 did not alter the electrode responses.

Basal measurements were made on embryos between the 2-cell and 64-cell stages. The mean value for cytoplasmic Ca^{2+} was pCa 6.5 (0.32 μM) $\pm$ 0.12 (mean $\pm$ s.e.m.) from 12 reliable

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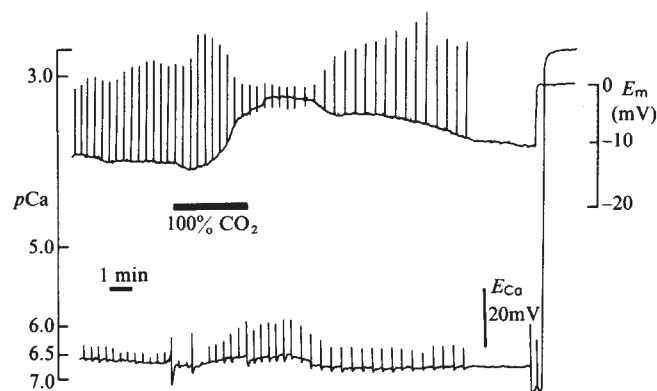


Fig. 1 Membrane potential, electrical coupling and intracellular free Ca^{2+} in a 16-cell *Xenopus* embryo during exposure to 100% CO_2 . Three intracellular microelectrodes were used; one voltage recording electrode and one Ca^{2+} -sensitive electrode in one cell, and a third electrode in an adjacent cell which injected repetitive, rectangular current pulses (10 nA, 1 s duration). Upper trace gives the membrane potential, the lower trace the potential E_{Ca} , measured as the difference between the potentials recorded by the voltage and Ca^{2+} -sensitive electrodes. Electrotonic potentials produced by current injected into the adjacent cell are shown as upward deflections on the membrane potential record. The height of these deflections gives a simple indication of the degree of current spread from cell to cell. The changes in input and junctional resistances during CO_2 treatment have been documented^{6,7}. The deflections on the lower trace reflect capacitative artefact which is picked up to a greater degree by the Ca^{2+} electrode because of its long electrical time constant. The frequency of the injected current pulses was reduced during the latter part of the trace to improve resolution of baseline E_{Ca} . The right-hand vertical axis shows the membrane potential. The left-hand vertical axis shows the *in vitro* calibration for this Ca^{2+} electrode. All pCa calibrating solutions contained 0.1 M KCl. The required pCa was set by 5 mM Ca and 10 mM ligand, nitrilotriacetic acid for pCa 5.0, *N*-(2-hydroxyethyl)ethylenediamine-*N,N',N'*-triacetic acid (HEEDTA) for pCa 6.0 and 6.5, and EGTA for pCa 7.0. The solutions also contained 10 mM TAPS, HEPES, HEPES and MOPS respectively, and were titrated with KOH to the correct pH values (8.42, 7.70, 8.20 and 7.29) to give the desired pCa using stability constants from ref. 18. The embryo was continuously superfused with a Holtfreter solution containing 60 mM NaCl, 1 mM KCl, 1.8 mM CaCl_2 and 2 mM ACES, pH 6.5. During the period marked by the bar, the superfusion solution was changed to bicarbonate-buffered Holtfreter solution (40 mM NaHCO_3 replacing 40 mM NaCl) bubbled with 100% CO_2 . After 3 min of exposure to CO_2 , the cell had depolarised and the voltage deflections had completely disappeared, only a biphasic capacitative artefact remaining on the trace. The lower, E_{Ca} , trace shows a small increase in intracellular Ca^{2+} . On return to the initial Holtfreter solution electrical coupling and E_{Ca} recovered. The current pulses were stopped, and first the voltage and then the Ca^{2+} -selective electrode were withdrawn. The recording was deliberately terminated before the membrane potential had reached its original level to recalibrate the Ca^{2+} electrode as soon as possible after the baseline pCa level had been re-established.

solutions to try to set and maintain intracellular free Ca^{2+} levels (ref. 4 and below). Cells buffered to less than 10 nM Ca^{2+} did not divide, suggesting that the electrode measurements of $\sim 0.1 \mu\text{M}$ Ca^{2+} in uninjected, dividing cells is not a gross overestimate. Cells buffered at around 0.1 μM calcium did divide, suggesting that transients above this level are not essential.

Two experimental procedures reported to block the flow of electrical current via gap junctions from one cell to another are elevation of intracellular free Ca^{2+} (refs 2, 3) and intracellular acidification with CO_2 -rich external solutions^{6,7,13}. It is known that raising intracellular Ca^{2+} can produce a fall in intracellular pH (pH_i)¹⁴, and conversely in some preparations lowering pH_i may increase free Ca^{2+} (refs 5, 15). The question then arises whether Ca^{2+} and H^+ act independently on the gap junctions or whether one ion acts via the other. To try to examine this question directly, free Ca^{2+} levels were measured in 16- to 64-cell stage embryos during exposure to 100% CO_2 . This level of CO_2 almost invariably uncouples cells in these embryos^{6,7}. In a few experiments electrical coupling was monitored along with free Ca^{2+} ; in others the depolarisation known to accompany the uncoupling was used to mark the effect of CO_2 on the cells. Figure 1 shows an experiment on a 16-cell embryo with an initial pCa of 6.7. On exposure to 100% CO_2 (with almost constant external pH), the cell depolarised and current flow from cell to cell was abolished. The pCa increased to about 6.45 (free Ca^{2+} elevation from 0.2 μM to 0.35 μM). After removal of the CO_2 these changes reversed. Similar small elevations of pCa were seen in other experiments. In six trials the mean initial pCa was 6.4 ± 0.13 ($\pm$ s.e.m.) and during exposure to 100% CO_2 the mean peak level of pCa was 6.19 ± 0.1 (0.63 μM). Thus lowering pH_i from about 7.8 to 6.5 (ref. 7) modestly raises free Ca^{2+} to less than 1 μM . The measurement of these small elevations in free Ca^{2+} relies on the virtual insensitivity at pCa 6–7 of the electrodes to pH changes over the experimental range, a technical advantage not shared by other techniques in use^{5,16,17}. The source of the Ca^{2+} transient has yet to be established. Some Ca^{2+} could enter the cells across the plasma membrane from the

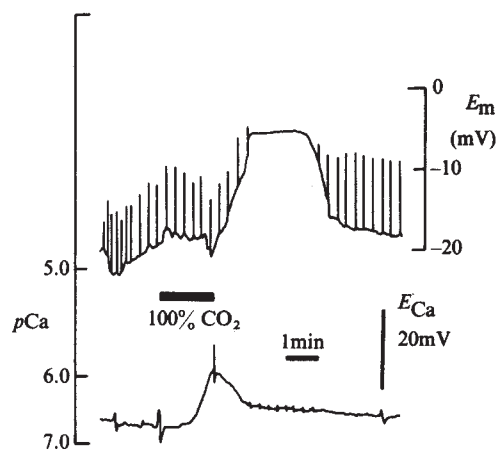


Fig. 2 Effect of 100% CO_2 on electrical coupling, membrane potential and E_{Ca} in one cell of an embryo at the 32-cell stage which was preinjected with CaEGTA. All experimental conditions were as in Fig. 1. A small amount, about 5% of the cell volume, of 200 mM CaEGTA solution containing some fast green FCF stain was injected into one half at the 2-cell stage. The injected half was recognised at later stages of development by the green stain and by the changes in pigment distribution which occurred during CO_2 treatment only in the injected half. Before withdrawing the electrode this embryo was again challenged with 100% CO_2 (not illustrated). This produced a slightly smaller and less prompt rise in pCa, but the cells did not uncouple. The absence of uncoupling may have been because the embryo suffered cumulative damage as a result of simultaneously raising Ca^{2+} and H^+ . Normal embryos (not injected with CaEGTA buffer) uncouple repetitively in response to successive changes with 100% CO_2 (ref. 7).

impalements in 10 embryos from 7 different batches. The lowest Ca^{2+} levels in this series were pCa 7.3 and 7.1. As most of the errors and artefacts in this type of experiment will lead to elevations in free Ca^{2+} , the value in undisturbed cells is likely to be in the region of 0.1 μM . This is in line with values for free Ca^{2+} obtained in a variety of other cells using all available techniques^{10–12}. It contrasts with the ~ 7 mM total Ca^{2+} in these embryos⁹. Free Ca^{2+} in the intercellular fluid, which lies in the blastocoel cavity, came to 1.12 ± 0.04 mM ($\pm$ s.e.m., $n = 9$).

No changes in free Ca^{2+} were detected during the cell cycle; in some cases the electrodes remained in one cell throughout two complete cleavages. Further evidence for the measured pCa level and lack of transients comes from complementary experiments in which eggs were injected with calcium buffer

blastocoel fluid; equally acidification may release Ca^{2+} from intracellular binding sites. It is even possible that CO_2 might release part of the bound Na^+ of these cells⁹, which might then contribute to the electrode response. If so, then the true Ca^{2+} transient would be even smaller than the figures quoted above.

Could the CO_2 have induced larger elevations of Ca^{2+} that escaped detection? To check that large transients could be seen, free Ca^{2+} levels were measured in embryos which had been injected with 200 mM CaEGTA (1:1 Ca to EGTA) to a final concentration of 5–10 mM. The injection was made into one cell of a two-cell embryo, which was then left to cleave to the 32- or 64-cell stage. At the normal pH_i of 7.8, almost all the injected Ca^{2+} would stay complexed to the EGTA as the effective dissociation constant was 10 nM (ref. 18). The CaEGTA then would have very little buffering power and could hardly change the free Ca^{2+} inside the cell. On acidification to, say, pH 6.5, the effective dissociation constant would rise to 4 μM , high enough for much of the Ca^{2+} to be unloaded and for the EGTA now to contribute significantly to the total calcium buffering inside the cell. The free Ca^{2+} should go to some value near 4 μM (pCa 5.4) depending on the relative buffer capacities of the cytoplasm and the injected EGTA. Figure 2 shows an experiment of this type. On the application of 100% CO_2 there was a marked, abrupt increase in free Ca^{2+} from pCa 6.6 to 5.9 (0.25 to 1.25 μM). In seven such trials the mean initial and peak pCa values were 6.28 ± 0.16 and 5.76 ± 0.06 . This latter value is reasonably close to the pCa 5.4 effective dissociation constant of the EGTA, considering that the intracellular Ca^{2+} buffering is not quantitated and that the change in pH_i is somewhat variable⁷. Clearly the electrodes are able to record large, experimentally induced elevations of free Ca^{2+} . As shown in Fig. 2 the application of CO_2 depolarised and uncoupled the cells; the Ca^{2+} transient was ending as the uncoupling began.

It seems, then, that 100% CO_2 produces elevations of free Ca^{2+} well below the ~50- μM level^{2,19} considered to be necessary to abolish current flow from cell to cell. Again one could ask if CO_2 might have caused Ca^{2+} increases localised in every experiment far from the tip of the Ca^{2+} electrode. Two lines of evidence argue against such a possibility. First, Rose and Rick⁵ have examined the effect of CO_2 on the luminescence of aequorin microinjected into *Chironomus* gland cells. In the 40% of cells which showed an increased glow, that increase was diffusely distributed. Second, we have injected *Xenopus* embryos with a new Ca^{2+} buffer, 1,2-di(*o*-amino-phenoxy)ethane-*N,N,N',N'*-tetraacetic acid²⁰, whose affinity for Ca^{2+} (log stability constant = 6.97) is practically independent of pH and Mg^{2+} in the physiological range. The final concentrations of ~10 mM buffer half-saturated with Ca^{2+} should have clamped the intracellular calcium near 0.1 μM . Of 45 such embryos, 64% continued to cleave, although often at a slower than normal rate. In 4 out of 5 injected embryos, the cells uncoupled when challenged with 60% or 100% CO_2 . These experiments suggest that *Xenopus* embryos can still be uncoupled when Ca^{2+} transients are greatly damped.

Thus, our results show the levels of free Ca^{2+} in cells of the early amphibian embryo to be in the region of 0.1 μM . They suggest that cell division is not accompanied by transient changes in free Ca^{2+} . A minimum level of Ca^{2+} seems to be necessary for cell division to occur⁴, but Ca^{2+} does not have a regulatory role in these cells. The results also support the view that CO_2 -induced uncoupling of cells in this preparation is not mediated by alterations in free Ca^{2+} levels. However, the relative importance of Ca^{2+} and H^+ in the control of gap junction permeability may vary from tissue to tissue²¹.

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Host gene influences sensitivity to interferon action selectively for influenza virus

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Several examples of genetically determined resistance to different viruses have been studied in mice¹. Inborn resistance due to the allele *Mr* in A2G mice may be characterised as follows: resistance is specific for members of the orthomyxo family (influenza virus)²; it is expressed in different organs and cells³; and it is independent of a functioning immune system^{3,4,7}. The only means of rendering resistant mice fully susceptible to influenza virus has been treatment with potent anti-interferon serum⁸, indicating that interferon is important in resistance. Interferon is generally thought to inhibit the multiplication of all animal viruses relatively indiscriminately. Therefore, it had seemed unlikely that interferon was involved in this resistance, as A2G mice were selectively resistant to influenza virus albeit as sensitive as control mice to several other viruses⁴. There are, however, examples in which the genetic control of the production of interferon or the sensitivity to interferon action is expressed selectively for a given virus. Thus, De Maeyer and coworkers have shown that four different *If* loci control the amount of circulating interferon produced after injection of mice with Newcastle disease virus (NDV)⁹, mouse mammary tumour virus¹⁰, or Sendai virus (personal communication). Hanson *et al.*¹¹ found that cultures of cells from C3H RV mice, resistant to Arbo B virus infection, were much more susceptible to the inhibitory effect of interferon when tested with Arbo B viruses than cultures taken from susceptible C3H mice. We present here results, showing that cultures of macrophages from mice resistant to orthomyxoviruses (*Mr* positive) can be protected by much smaller amounts of interferon than are necessary to protect cultures from susceptible mice when these cultures are infected with influenza virus; whereas no difference in sensitivity to interferon is observed when cultures are infected with vesicular stomatitis virus (VSV) or encephalomyocarditis virus (EMC). We believe that these results showing a genetic control of sensitivity to interferon action specific for a given virus are related to the *in vivo* resistance of A2G mice to influenza virus. If applicable to man, these results may also be of importance in understanding individual variations in sensitivity to viral infections.

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Table 1 Viral susceptibility of cultured macrophages

Macrophage		Titre of virus (log ₁₀ TCID ₅₀ per ml)		
Origin	Genotype	M-TUR	EMC	VSV
CBA/J	(+/+)	8.3	7.8	8.5
A/J	(+/+)	7.8	ND	9.0
A2G	(Mx/Mx)	7.8	7.8	8.8
(A2Gx A/J)F ₁	(Mx/+)	8.3	ND	ND

A/J and CBA/J mice were from G. L. Bomholtgård. Inbred A2G mice, homozygous for the resistance allele *Mx*² and heterozygous (A2G × A/J)F₁ hybrids were bred locally. Macrophage cultures were prepared as described previously^{6,8}. Briefly, peritoneal macrophages from 8- to 12-week-old mice stimulated 3 days previously by intraperitoneal (i.p.) injection of 2 ml of thioglycollate medium were collected. 1.5 × 10⁶ viable cells in 1.0 ml of RPMI 1640 medium were plated into 16-mm wells of 24-well tissue culture cluster plates. After frequent washings to remove non-adherent cells, macrophages were incubated for 4 days in serum-free medium. Thereafter, RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum was added and adherent cells were kept in culture for a total of 21 days. Macrophage cultures were then washed and 0.5 ml of serial 10-fold viral dilutions in serum-free culture medium were added to quadruplicate wells. Cytopathic effects for the following three viruses were assessed 72 h after infection: *M-TUR*, an avian influenza A virus strain derived from A/Turkey/England/63 (Hav₁, Nav₃)¹⁷ adapted to grow in mouse peritoneal macrophages *in vitro*⁶. Stock allantoic fluid had a haemagglutination titre of 1:640 and 10^{8.7} mean tissue culture infective doses (TCID₅₀) per ml when assayed on primary chick embryo fibroblast (CEF) cultures. *VSV*, serotype Indiana was grown and stored as previously described¹⁵. *VSV* stock allantoic fluid had a titre of 10^{8.7} TCID₅₀ per ml on L₉₂₉ cells. *EMC* virus¹⁸ was propagated in monolayer cultures of L₉₂₉ cells and was adapted to grow in mouse macrophages by five consecutive passages in the murine macrophage monocytic tumour cell line J-774 (ref. 19) followed by two passages in peritoneal macrophages from CBA/J mice cultured for 3 weeks. Virus stocks consisted of cell free medium from 24-h cultures of J-774 cells and had a titre of 10^{8.5} TCID₅₀ per ml for L₉₂₉ cells. ND, not determined.

Macrophages collected from the peritoneal cavity of A2G mice are resistant to influenza virus when first placed in culture⁶, but become susceptible with time⁸. We therefore cultivated peritoneal macrophages for 3–4 weeks, at which time they were highly susceptible to the three test viruses used, macrophage-adapted orthomyxovirus strain (M-TUR), a rhabdovirus (VSV) or a picornavirus (EMC) (Table 1). At 3 to 4 weeks, cultures of macrophages were incubated with graded doses of mouse interferon for 18 h before viral challenge. Two preparations of mouse interferon induced by NDV in mouse C-243 cells were used: (1) partially purified to 1 × 10⁷ reference units per mg protein¹²; (2) highly purified by sequential chromatography on sheep anti-interferon antibody¹³ and CM-sepharose¹⁴ columns to 5 × 10⁸ reference units per mg protein (a gift from Dr M. Aguet).

Figure 1 illustrates the effects of 40 and 400 reference units of partially purified interferon on the multiplication of influenza virus M-TUR in macrophages from CBA/J (susceptible) and A2G (resistant) mice. At high (Fig. 1 *a, b*) and low (Fig. 1 *c, d*) multiplicities of infection, both interferon doses markedly inhibited virus replication in A2G mouse macrophage cultures (Fig. 1 *b, d*), whereas even 400 interferon units exerted only a slight inhibition or delay of viral multiplication in CBA/J macrophage cultures (Fig. 1 *a, c*).

Figure 2 shows the effect of different amounts of highly purified interferon on the multiplication of M-TUR virus and EMC virus in cultures of macrophages from (A2G × A/J)F₁ mice (heterozygous for the gene *Mx* and thus genetically resistant to influenza virus but susceptible to EMC virus²) and from A/J mice (susceptible to both viruses). The inhibitory effect of interferon on the multiplication of EMC virus was similar in cultures of macrophages from these two strains of mice. In contrast, a marked difference was observed in the degree of inhibition by interferon of the multiplication of M-TUR virus in

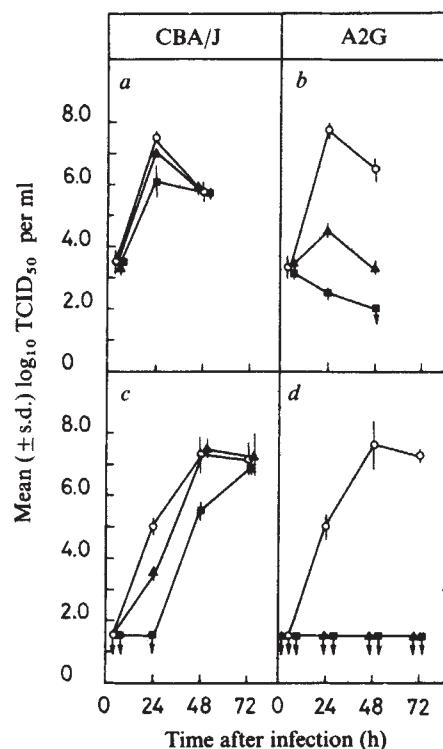


Fig. 1 Inhibition by interferon of multiplication of M-TUR virus in cultures of macrophages from susceptible CBA/J mice (*a, c*) or resistant A2G mice (*b, d*). Peritoneal macrophages cultivated for 3 weeks in culture were incubated for 18 h with 40 (▲) or 400 (■) reference units per ml of partially purified mouse interferon. Interferon pretreated and untreated cells (○) were then washed and infected with M-TUR virus at multiplicities of either 5 (*a, b*) or 0.005 (*c, d*). After incubation for 60 min at 37 °C the virus inoculum was removed by repeated washings. Cell-free medium from individual cultures was assayed for infectivity on CEF cultures at the times indicated. Each point indicates the mean TCID₅₀ from three cultures with standard deviation.

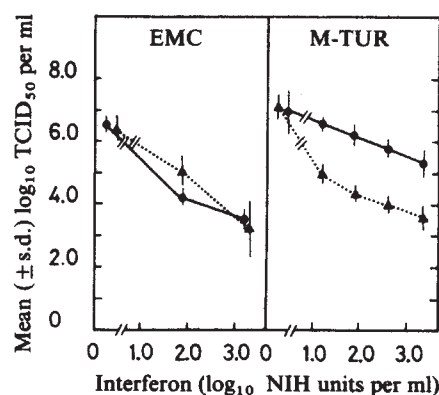


Fig. 2 Effects of highly purified interferon on multiplication of EMC and M-TUR virus in macrophages from resistant or susceptible mice. Peritoneal macrophages from resistant (A2G × A/J)F₁ mice (Mx/+), (▲ ··· ▲), and from susceptible A/J mice, (+/+), (● ··· ●), were cultured for 3 weeks and incubated for 18 h with various doses of highly purified interferon. Thereafter, cultures were washed and infected at a multiplicity of 5 with either M-TUR or EMC virus. After an incubation period of 60 min at 37 °C the virus inoculum was removed by repeated washings. The viral titre of cell-free medium from four individual cultures per interferon dilution was determined 18 h after viral infection (at the time of peak virus production in control cultures). EMC virus was titred in monolayer cultures of L₉₂₉ cells and M-TUR virus in CEF. Mean TCID₅₀ values and standard deviations are indicated. The differences in yields of M-TUR virus between interferon-treated +/+ and Mx/+ macrophage cultures were statistically significant (*P* < 0.005 for all interferon doses used).

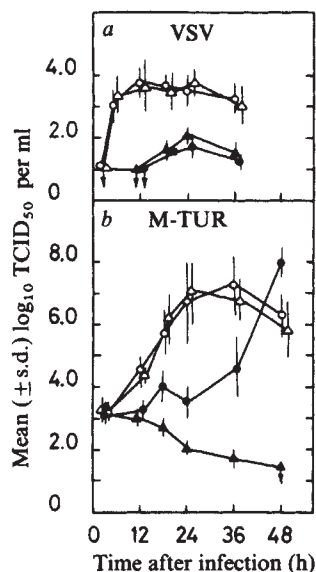


Fig. 3 Effect of interferon on the multiplication of M-TUR and VSV in macrophage cultures from backcross mice segregating for the allele *Mx*. (A2G×C57BL/6J)_{F₁} were backcrossed with C57BL/6J mice. The offspring were characterised as carriers (*Mx*/+) or noncarriers (+/+) of the allele *Mx* as described⁶. After 3 weeks in culture, peritoneal macrophages obtained from individual *Mx*/+ or +/+ backcross mice were incubated for 18 h with 20 (a) or 40 (b) reference units of partially purified mouse interferon. Cells were then washed and infected with either VSV (a) or M-TUR (b) at a multiplicity of 5. After incubation for 90 min at 37°C the virus inoculum was removed by repeated washings. Cell-free medium from individual cultures was titrated on CEF monolayers at the times after infection indicated. Titres represent mean values from three macrophage cultures of either resistant (*Mx*/+) or susceptible (+/+) genotype. ○, (+/+) untreated; ●, (+/+) interferon-treated; △, (*Mx*/+) untreated; ▲, (*Mx*/+) interferon-treated.

macrophage cultures from these mice. Thus in A/J cells, 2,000 reference units of interferon resulted in only a 50-fold reduction in the yield of M-TUR virus, whereas the same amount of interferon reduced M-TUR virus yield by 4,000-fold in (A2G×A/J)_{F₁} cells.

In backcrosses segregating for the two alleles at the *Mx* locus, the susceptibility (+/+) or resistance (*Mx*/+) to orthomyxoviruses of individual mice can be determined by typing peritoneal macrophages⁶. Interferon had a similar and profound inhibitory effect on the multiplication of VSV in both *Mx*/+ and +/+ cells (Fig. 3a) (*Mx*/+ and +/+ mice are equally susceptible to VSV)¹⁵. In contrast, interferon slightly delayed multiplication of M-TUR virus in +/+ cells, but markedly inhibited its multiplication in *Mx*/+ cells (Fig. 3b).

We have not yet determined which step in influenza virus replication is affected in interferon treated *Mx*-bearing cells. Our preliminary results indicate that neither virus adsorption nor virus penetration are involved but that viral protein synthesis seems to be blocked at an early stage (M. Horisberger *et al.*, in preparation).

Most laboratory strains of mice do not carry the *Mx* gene, and thus support influenza virus multiplication despite exposure to relatively large amounts of interferon. However, there are indications that resistance of wild mouse populations to orthomyxoviruses is widespread (O.H., unpublished). We do not know whether this resistance is also interferon mediated (as in our A2G mice) but it is of interest that interferon also seems to be involved in mouse resistance to flaviviruses¹¹, and that wild mice are also frequently found to be resistant to these viruses¹⁶. The phenomenon of genetic resistance to viral infections mediated by interferon may therefore be more general than has heretofore been supposed. It is an interesting example of cooperation between a nonspecific extrinsic factor (interferon)

with an intrinsic element (the resistance gene) resulting in a degree of antiviral specificity. Thus, the resistance gene can be envisaged either as modulating the antiviral state induced by interferon or as coding for an antiviral mechanism activated by interferon. Alternatively, it might be speculated that the seemingly nonspecific antiviral state generally observed in interferon-treated cells would be composed of a multitude of specific antiviral mechanisms each of which might be coded for by a unique resistance gene of the host cell. Allelism at such resistance loci would then account for various degrees of protection induced by interferon.

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Spontaneous stomach ulcer in genetically mast-cell depleted *W/W^o* mice

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Histamine has been implicated in the pathogenesis of gastroduodenal ulcers^{1,2}. Since mast cells contain a considerable amount of histamine as well as heparin and other biologically active substances³, it seems reasonable to speculate that they are involved in this process^{4,5}. In fact, development of gastroduodenal ulcers was reported in dogs with mastocytoma⁶, and an increase in mast-cell number was described in the vicinity of human gastroduodenal ulcers^{7,8}. Y.K. *et al.*⁹ have recently found that the number of mast cells in a unit length of the skin of *W/W^o* mutant mice is less than 1% of the value for the congenic +/+ mice and that no mast cells are detected in the intestinal canal of *W/W^o* mice. Therefore, this mutant mouse was used to investigate the role of mast cells in the induction of gastroduodenal ulcers. As a preliminary experiment, we examined the stomach and duodenum of *W/W^o* mice without any treatments. Unexpectedly, we found the spontaneous development of perforating stomach ulcers in *W/W^o* mice, suggesting that mast cells do not necessarily have aggravating effects on the production of stomach ulcers.

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Table 1 Incidence of stomach ulcers in WBB6F₁ mice of different genotype, sex and age

		Incidence of ulcers in each age (no. (%))	
		1–2 months	2–6 months
W/W ^v	Male	9/18 (50)	11/24 (46)
W/W ^v	Female	21/28 (75)	4/16 (25)
W/+	Male and female	0/19 (0)	0/22 (0)
W ^v /+	Male and female	0/18 (0)	0/20 (0)
+/+	Male and female	0/23 (0)	0/21 (0)

Mice of WBB6F₁(WB-W/+ × C57BL/6-W^v/+)- (W/W^v, W/+, W^v/+, +/+) were used. WBB6F₁ mice were raised in our laboratory using parental stocks originally obtained from the Jackson Laboratory, Bar Harbor. The W genotype of all animals was inferred from the pattern of white spots and dilution of coat colour¹⁰. In addition to mast-cell depletion, the mice of W/W^v genotype show severe macrocytic anaemia, sterility and depletion of hair pigment¹⁰. Mice were kept in conventional animal facilities with *ad libitum* access to Oriental Lab Chow (Oriental Yeast Co.) and tapwater.

At an age of 1 to 6 months, W/W^v mutant mice and their littermates were killed; the abdominal wall was opened; deformity of pyloric region and adhesion between pylorus and adjacent organs were inspected. The stomach and duodenum were removed as a whole; the stomach was split along the greater curvature. After macroscopic examination, the stomach and duodenum were fixed in 10% buffered formalin (pH 7.2). Ordinary paraffin sections (5 µm thick) were made, and stained with haematoxylin and eosin to confirm diagnosis, or with toluidine blue to determine the number of mast cells, or with the periodic acid Schiff reaction to count the number of mucous cells. In most cases, subserial sections were made to determine the size of the ulcer when found. The diameter of the ulcer was measured in the largest cross-section of the ulcer using an ocular micrometer attached to an eyepiece of the microscope.

Gastric secretions were collected for 4 h by the method described by Shay *et al.*¹¹. The volume of secretion was measured, the acid concentration was titrated with 0.1 M NaOH, and the acid output was calculated. Pepsin concentration was determined according to the method reported by Anson and Mirsky¹². Histamine was separated by column chromatography after dansylation from various parts of the stomach and its concentration was determined with the fluorimeter¹³. Distribution of enterochromaffin-like cells was examined with histofluorescence technique¹⁴ with or without prior injection of L-dopa (100 mg per kg body weight) (ref. 15).

In about a half of the untreated W/W^v mice, chronic ulceration was found in the prepyloric region of the stomach. The incidence of ulcers was not affected by age in males, but was higher in young females (1–2 months) than in older ones (2–6 months) (Table 1). The size range of the ulcers was 0.6–4.5 mm and the median size was 2.2 mm. In some cases, the lesion was so

Table 3 Mast-cell number and histamine concentration in various parts of the stomach of WBB6F₁-W/W^v and the congenic +/+ mice

Part of stomach	No. of mast cells*† (per cm of section)		Histamine concentration* (nmol per g of wet tissue)	
	W/W ^v	+/+	W/W ^v	+/+
Forestomach	0 (9)	172 ± 16 (11)	0.6 ± 0.4 (4)	78 ± 16 (4)
Fundic region	0 (9)	139 ± 11 (11)	57 ± 7 (5)	123 ± 12 (3)
above muscularis mucosae	0 (9)	205 ± 1 (11)		
beneath muscu- laris mucosae	0 (9)	134 ± 11 (11)		
Prepyloric region	0 (9)	62 ± 8 (11)	15 ± 4 (4)	
above muscularis mucosae	0 (9)	25 ± 2 (11)		
beneath muscu- laris mucosae	0 (9)	37 ± 7 (11)		21 ± 2 (4)

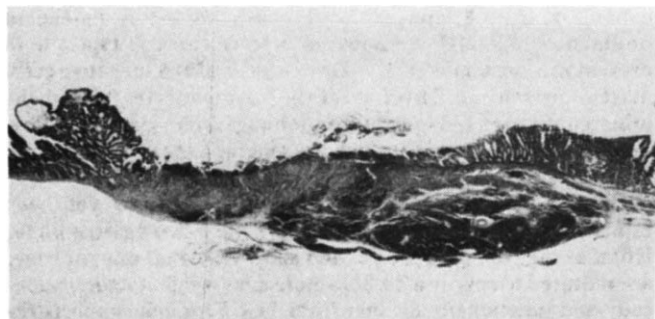
* Mean ± s.e., number of mice is shown in parentheses.

† See ref. 9 for the method used to determine the number of mast cells.

wide that it occupied the whole prepyloric region, but it did not extend into the fundic part even in such cases. The ulcer frequently perforated, and adhesion of the pyloric region to the liver and pancreas was observed. Histologically, the ulcer crater contained necrotic debris and was sharply demarcated and infiltrated by inflammatory cells. The muscular layers were interrupted by granulation tissue and fibrous connective tissue (Fig. 1). When the section was stained with toluidine blue, no mast cells were detected either in the vicinity of the ulcer or in other parts of the stomach and duodenum. In contrast with the high incidence of ulceration in mast-cell depleted W/W^v mice, no ulcerative lesions were detected in the stomach and duodenum of W/+, W^v/+, and +/+ littermates which have a considerable number of mast cells in the stomach (Table 1).

Since peptic ulcers are thought to occur as a consequence of an imbalance between the factors stimulating acid secretion and the factors protecting the gastric mucosa from acid/peptic digestion¹⁶, the numbers of parietal cells, chief cells and mucous cells were counted in the stomachs of the +/+ mice and those of W/W^v mice with or without stomach ulcers. As shown in Table 2, the numbers of parietal cells, chief cells and mucous cells in the +/+ mice did not differ significantly from the values observed in W/W^v mice with or without the ulcers. Moreover, when the gastric juice was examined, there was no difference in the rates of acid and pepsinogen secretion between the +/+ and W/W^v mice.

As the normal acidity was observed in the stomach of mast-cell depleted W/W^v mice and as histamine is known to be a stimulator of acid secretion¹⁶, the concentration of histamine was evaluated in the stomach of W/W^v and congenic +/+ mice. The number of mast cells was counted in various parts of the stomach to compare with the concentration of histamine. Since the concentration of histamine in the forestomach of the

**Fig. 1** Histological section of a chronic ulcer localised in the prepyloric region of the stomach of the untreated WBB6F₁-W/W^v mice (stained with haematoxylin and eosin).**Table 2** Number of various types of cells in the gastric mucosa of WBB6F₁-W/W^v and the congenic +/+ mice

Cell type	No. of cells per gland (mean ± s.e.)		
	W/W ^v with ulcer	W/W ^v without ulcer	+/+
Parietal cell	20 ± 3 (10)	18 ± 1 (15)	17 ± 2 (12)
Chief cell	15 ± 4 (10)	15 ± 2 (15)	12 ± 1 (12)
Surface mucous cell	23 ± 2 (10)	21 ± 1 (15)	21 ± 1 (12)
Neck mucous cell	7 ± 1 (10)	6 ± 1 (15)	7 ± 1 (12)
Pyloric mucous cell		33 ± 2 (7)	33 ± 1 (10)

Number of mice is shown in parentheses.

W/W^v mice was less than 1% of the value of the $+/+$ mice, the histamine content seemed to be proportional to the number of mast cells in the forestomach (Table 3). In contrast, a considerable amount of histamine was detected in the glandular stomach of mast-cell depleted W/W^v mice (Table 3), suggesting the presence of histamine in certain types of epithelial cells located in the glandular stomach of the W/W^v mice. Examination with the fluorescence microscope revealed that the distribution and number of enterochromaffin-like cells in the fundic mucosa of the W/W^v mice were not significantly different from those observed in the congenic $+/+$ mice. The prior injection of L-dopa increased the number of fluorescent cells in both W/W^v and $+/+$ mice.

The present results showed that the stomach ulcer developed spontaneously in mast-cell depleted W/W^v mice and that the glandular stomach of W/W^v mice contained a considerable amount of histamine in spite of its lack of mast cells. Since the number of enterochromaffin-like cells in the fundic mucosa of the W/W^v mice was not different from the value observed in the $+/+$ mice, histamine detected in the glandular stomach of the

W/W^v mice appears to originate from certain types of enterochromaffin-like cells as demonstrated by Håkanson *et al.*¹⁷. Therefore, there are possibilities that histamine derived from enterochromaffin-like cells may have an essential role in the pathogenesis of gastroduodenal ulcers as postulated by Guth and Code¹ and Lees *et al.*¹⁸ and that mast cells themselves might have inhibiting rather than aggravating effects on the production of ulcers.

The histological features of the ulcers found in the W/W^v mice are very similar to the chronic human ulcers¹⁶. Although production of acute ulcers in the fundic mucosa of rats by stress¹⁹ and spontaneous development of duodenal ulcers in NZB mice²⁰ were previously reported, development of chronic ulcers in the prepyloric region is rare in untreated rodents. Therefore, the ulcer in the W/W^v mice may be a useful model of the human disease.

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Two distinct factors are required for induction of T-cell growth

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The molecular and cellular basis of T-lymphocyte activation remains a central question in immunology. The growth of already proliferating T cells is known to depend on T-cell growth factor (TCGF), a physiological mitogen¹. Noncycling T lymphocytes, however, are not sensitive to TCGF². They require a short contact with mitogenic lectins, such as concanavalin A (Con A)³ or leukoagglutinin⁴ to bind and respond to TCGF, and will thereafter maintain exponential growth for long periods provided that TCGF is not limiting⁵. While the induction of TCGF reactivity results from the direct contact of Con A with resting T cells³, the lectin-dependent production of TCGF is known to involve two cell types, both present in mouse spleen^{1,3,6,7}. One consists of I-A-negative cells, most of which are Thy-1-positive T lymphocytes^{1,3}, and the other consists of I-A-positive, immunoglobulin-negative, Thy-1-negative cells, most of which are macrophages^{1,3}. The nature of the respective contributions of the two cell types, and in particular the cellular origin of TCGF, has not yet been established. We have now established the I-A-negative population as the source of TCGF and show here that macrophages are required to supply a 20,000-molecular weight factor, chemically and functionally distinct from TCGF, which supports the production of TCGF by the I-A-negative cells.

TCGF was recovered in conditioned medium from Con A stimulated mouse spleen cell cultures⁵, and assayed by its

capacity to maintain proliferation of T-cell blasts^{1,8}. These were derived from spleen cell cultures initially stimulated by Con A and subsequently passaged in CM for periods of 3 weeks or more. Where indicated, a cloned TCGF-dependent cell line of specific cytotoxic T lymphocytes was also used⁹. When TCGF from Con A conditioned medium was partially purified by isoelectric focusing and then fractionated on Sephadex, TCGF activity was detected in fractions corresponding to an apparent molecular weight (MW) near 35,000 (Fig. 1). Identical activity profiles were obtained for fractions tested on the two types of T-cell blasts and on resting I-A-negative T cells after a 4-h sensitising pulse of Con A. The active fractions were pooled and used as a source of partially purified TCGF in subsequent experiments.

The proliferative responses of T cells to Con A were then examined in spleen cell populations depleted of I-A-positive cells. As previously found^{1,3,7}, Con A alone failed to induce proliferation (Table 1). We therefore tested medium conditioned by macrophages for its capacity to replace the I-A-positive cell requirement. The results shown in Table 1 were obtained with a partially purified preparation of medium conditioned by WEHI-3 cells. These are continuously growing cells of BALB/c origin with functional markers characteristic of macrophages¹⁰. This material supported a strong proliferative response in the presence of Con A, but it was inactive alone. In these culture conditions, partially purified TCGF seemed to exert a similar action, supporting a response in the presence, but not the absence, of Con A. The combination of TCGF and WEHI-3 activity, however, was inactive in the absence of Con A, demonstrating that the WEHI-3 activity cannot replace Con A in its role of sensitising T cells to TCGF.

Although the WEHI-3-derived activity and TCGF behaved similarly in cultures of resting, I-A-negative cells, their distinct functional properties were apparent in cultures of already proliferating, TCGF-dependent T-cell blasts (Table 1). In such cultures, only TCGF was able to sustain proliferation. WEHI-3 activity had no inhibitory effect in combination with TCGF.

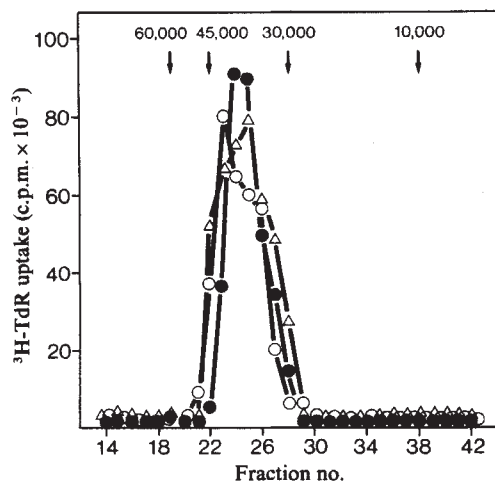


Fig. 1 Growth requirement of T-cell blasts and Con A pulsed purified T cells. Conditioned medium (CM) was obtained by incubating unseparated mouse spleen cells (5×10^6 per ml) for 24 h in the presence of concanavalin A (Con A) ($5 \mu\text{g ml}^{-1}$) (Pharmacia) in RPMI-1640 medium containing neomycin ($50 \mu\text{g ml}^{-1}$), HEPES (10 mM), 2-mercaptoethanol (5×10^{-5} M) and 1% fetal calf serum (GIBCO, batch U18710.11). CM (500 ml) was concentrated by dialysis against polyethylene glycol (MW 20,000, Serva), dialysed against buffered saline solution (BSS) and filter-sterilised ($0.45 \mu\text{m}$). Preparative flat-bed isoelectric-focusing (LKB) in a pH gradient from 3.0 to 10.5 was carried out over 24 h at constant power in Ultradex gel (LKB)²⁰. Fractions were collected, eluted from small columns, dialysed against BSS, and tested for TCGF activity on T-cell blasts. The TCGF-containing fractions (pH 6.0–7.0) were pooled, lyophilised, dissolved in the running buffer, applied to a calibrated Sephadex G-75 superfine column (1.6×90 cm), and eluted with 4 mM HEPES containing 0.16 M NaCl and $10 \mu\text{g ml}^{-1}$ of polyethylene glycol (MW 6,000), pH 7.3. Fractions were tested for TCGF activity on Con A-derived T-cell blasts (5×10^4 per ml) (●), a cloned anti-HY (H-2^b) killer cell line¹⁴ (5×10^4 per ml) (○) and I-A-negative resting T cells (2×10^5 per ml) (see legend to Table 1) which had been pulsed with Con A for 4 h (Δ). The proliferative responses were measured on day 4 and each point represents the mean value of ^3H -TdR uptake into DNA in triplicate cultures.

The chemical distinction between TCGF and the WEHI-3-derived activity became apparent when the latter was chromatographed on Sephadex (Fig. 2). Confirming the results in Table 1, no TCGF-like activity was observed in cultures of T-cell blasts with or without Con A (upper panel). However, activity was apparent when tested in cultures of I-A-negative populations containing resting T lymphocytes. There, fractions corresponding to an apparent MW near 20,000, inactive by themselves, supported Con A-dependent proliferation. Thus, TCGF maintains proliferation of T-cell blasts and has an apparent MW near 35,000 on Sephadex. In contrast, the activity from WEHI-3 cells has an apparent MW near 20,000, and is active in cultures of resting T cells but not on Con A-derived T-cell blasts.

These results suggested the possibility that the WEHI-3 product might support the T-cell response to Con A by allowing production or release of TCGF in the I-A-negative cell cultures. TCGF, in turn, would initiate and maintain T-cell growth. To determine whether this was the case, I-A-negative spleen cells were cultured with Con A and the partially purified WEHI-3 activity. After 24 h, the supernatant medium was collected and tested for TCGF activity in cultures of T-cell blasts. The results (Fig. 3) confirm that in the presence of both Con A and the WEHI-3 product, but not with either alone, I-A-negative cells are able to release abundant TCGF activity into the medium. Note that TCGF-dependent T-cell blasts, in contrast to unselected, resting splenic T lymphocytes, are not capable of producing TCGF (or maintaining proliferation) even in the

presence of both Con A and the WEHI-3 product. This observation confirms previous negative findings when TCGF-dependent T-cell blasts were incubated with splenic macrophages and Con A (ref. 1).

These experiments show that a soluble macrophage product with an apparent MW near 20,000 is required for Con A-induced elaboration of TCGF. This product is probably identical with that known as lymphocyte activating factor (LAF). This principle is defined as a macrophage-derived factor with an apparent MW near 15,000 which is detected by its capacity to support a proliferative response of thymocytes to low, non-mitogenic concentrations of T-cell lectins such as Con A (ref. 11). LAF is known to be secreted by several macrophage cell lines including WEHI-3 (ref. 12). In this context, the data in Table 1 can be regarded as confirming earlier indications that LAF can substitute for the macrophage requirement in mitogen-dependent T-cell proliferation¹³. Also, our observations confirm and extend those of Smith and coworkers, showing that crude conditioned medium from a macrophage cell line enhanced TCGF production by non-adherent spleen cells in response to Con A (ref. 14). In agreement, we regard the requirement for adherent, I-A-positive cells in T-cell proliferative responses to be now resolved into a requirement for molecules, probably already known as LAF, which are needed only for events leading to TCGF production. Although LAF release can be induced by several macrophage-stimulating agents¹⁵, the nature of the Con A-dependent interaction of T cells and macrophages which leads to production of LAF requires further investigation.

In addition, our results show that TCGF derives from I-A-negative cells rather than macrophages. These observations, together with those of Wagner and Rollinghoff¹⁶ indicating involvement of Lyt-1-positive (helper) T lymphocytes in the generation of TCGF, lead us to favour the view that TCGF originates from these cells. However, our methods do not exclude the possibility that TCGF actually originates from the 3% minority contaminant of I-A- and Thy-1-negative cells, with

Table 1 Growth induction in cultures of purified resting T cells and of T-cell blasts

Addition to test culture	Responses (c.p.m. per culture $\times 10^{-3} \pm \text{s.d.}$) of anti-I-A-treated and Ig-anti-Ig column passed spleen cells*	Responses (c.p.m. per culture $\times 10^{-3} \pm \text{s.d.}$) of T-cell blasts†
None	1.3 ± 0.3	0.9 ± 0.4
Con A	2.1 ± 0.9	0.8 ± 0.3
Con A + WEHI-3 activity	31.7 ± 3.6	1.5 ± 0.7
WEHI-3 activity	0.6 ± 0.3	1.2 ± 0.4
Con A + TCGF	19.7 ± 3.6	16.1 ± 2.9
TCGF	2.1 ± 0.5	17.3 ± 2.0
TCGF + WEHI-3 activity	1.7 ± 0.4	19.7 ± 3.3

* Spleen cell suspensions from C3H/HeJ mice were treated with monoclonal anti-I-A^k antibodies¹⁸ for 30 min in the cold and passed over a column of plastic beads coated sequentially with mouse Ig and rabbit anti-mouse Ig (ref. 19). The effluent cells, now depleted of I-A-positive and Ig-positive cells, were cultured at 2×10^5 per ml in microtitre plates (0.2 ml per culture), either alone or in the presence of the following reagents as indicated: Con A ($5 \mu\text{g ml}^{-1}$), partially purified activity from WEHI-3 conditioned medium (see Fig. 2 legend) and partially purified TCGF (see Fig. 1 legend). Mean values of triplicate cultures $\pm$ s.d. of incorporated ^3H -TdR on day 4 of culture are presented.

† T-cell blasts (5×10^4 per ml), which had been maintained in standard CM for more than 3 weeks, were tested in the same conditions as above. The results from day 3 of culture are presented.

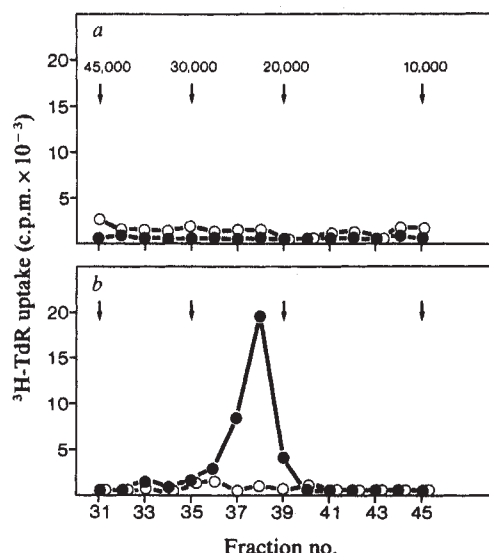


Fig. 2 Fractionation of the growth supporting activity in WEHI-3 CM. WEHI-3 CM was obtained from 5–7-d cultures of WEHI-3 cells in Iscove's modified Dulbecco's medium (IMDM) containing bovine serum albumin (BSA) ($750 \mu\text{g ml}^{-1}$), human transferrin ($30 \mu\text{g ml}^{-1}$) and soybean lipid ($50 \mu\text{g ml}^{-1}$) as described previously¹⁴. The WEHI-3 CM (760 ml) was concentrated by dialysis against polyethylene glycol (MW 20,000, Serva), applied to a Con A-Sepharose column ($2.5 \times 40 \text{ cm}$) (Pharmacia) and eluted with 50 mM Tris HCl pH 7.4 containing 0.5 M NaCl and 10^{-4} M Ca^{++} , Mg^{++} and Mn^{++} . The non-interactive material was concentrated over an Amicon Diaflo UM-10 membrane and thereafter applied to a Sephadex G-150 column ($2.5 \times 95 \text{ cm}$). Running buffer was as given in Fig. 1 legend. The growth-supporting activity was recovered in the post-albumin fractions, which were pooled and used in the experiments presented in Table 1. This pool was thereafter concentrated over a UM-10 membrane and applied to a Sephadex G-75 superfine column ($1.6 \times 90 \text{ cm}$) in the same running buffer as above. The fractions eluted from this column were tested for their capacity to support growth of *a*, T-cell blasts in the presence (●) or absence (○) of Con A ($5 \mu\text{g ml}^{-1}$); and *b*, purified resting T cells (see legend to Table 1) in the presence (●) or absence (○) of Con A ($5 \mu\text{g ml}^{-1}$). The proliferative responses were measured and each point represents the mean value of DNA-incorporated ^3H -TdR from triplicate cultures.

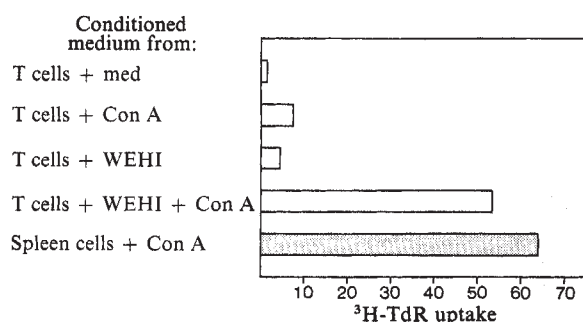


Fig. 3 WEHI-3 activity supports the Con A dependent production of TCGF by purified T cells. Spleen cells depleted of I-A-positive cells were cultured at 2.5×10^6 per ml either alone or with Con A ($5 \mu\text{g ml}^{-1}$) or partially purified WEHI-3-derived activity (pooled active fractions indicated in Fig. 2). The supernatant medium was tested for TCGF activity in T-cell blast cultures. As a positive control, normal spleen cells were cultured at 5×10^6 per ml in the presence of Con A ($5 \mu\text{g ml}^{-1}$) for 24 h. The proliferative responses were measured on day 3 of culture and the results are presented as the mean values of ^3H -TdR (c.p.m. $\times 10^{-3}$) incorporated into DNA in triplicate cultures.

T lymphocytes having some other essential role. Thus, the nature of the lectin and LAF interactions with these cells leading to the release of TCGF remain unclarified. Finally, as TCGF-dependent T-cell blasts, which include killer T cells^{5,9,17}, are not competent to product TCGF, it seems that different T-cell subpopulations are involved in production and response to TCGF. Although the requirement for TCGF by killer T lymphocytes is clear, a fuller knowledge of the requirements of the T helper cell population is needed.

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Immunisation against heterologous type II collagen induces arthritis in mice

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The induction of polyarthritis in rats by intradermal immunisation with homologous or heterologous type II collagen in complete or incomplete Freund's adjuvant was reported recently by Trentham *et al.*¹. We have now produced a similar disease in certain strains of mice.

Mice were injected with either type I or type II bovine collagen, prepared using the method of Trentham *et al.*², emulsified in complete Freund's adjuvant (CFA) or incomplete Freund's adjuvant (ICFA), in experimental conditions similar to those known to be effective in the rat. The time of onset, and incidence of arthritis were determined by daily clinical examination, regular measurement of the thickness of hind foot pads with a micrometer gauge, and after necropsy, by histological examination of proximal and distal joints in fore and hind limbs, the tail, ear and spinal column.

The findings reported here mainly concern the induction of arthritis, clinically affecting several joints, in the DBA/1 strain of inbred mice (Laboratory Animals Centre, Carshalton). Mice aged 8–15 weeks were initially injected intradermally in the back with bovine type I or type II collagen, or the acetic acid solvent for the collagens, emulsified in CFA or ICFA. Mice were challenged 3 weeks later with an intraperitoneal injection of the same type of collagen, or of acetic acid alone.

The first sign of swelling and redness of a fore or hind paw was detected after 23 d in mice injected with type II bovine collagen in CFA. The swelling progressed to a maximum at different times in individual joints and then gradually regressed, although the joints still appeared badly damaged on histological

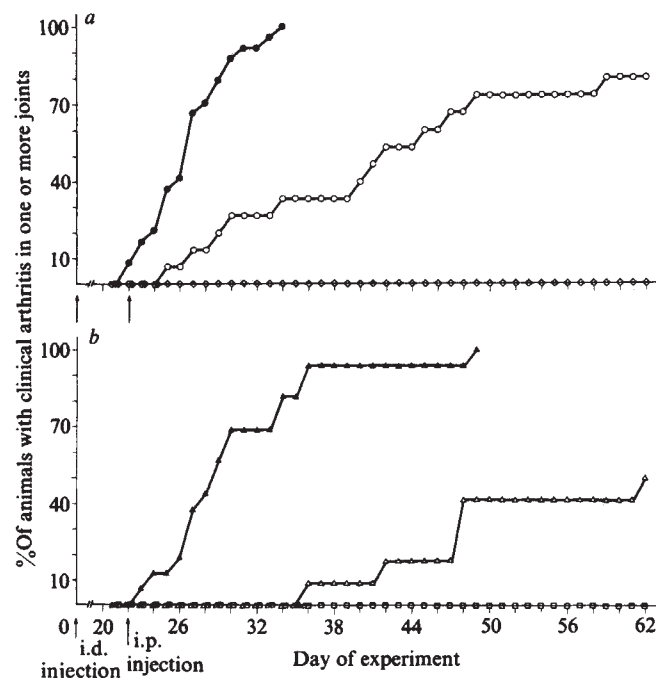


Fig. 1 Incidence and time of onset of arthritis in DBA/1 mice. DBA/1 mice were injected intradermally into 4–6 sites on the back with 100 μ g of bovine type II collagen, dissolved in 0.1 ml of 0.1M acetic acid and emulsified in an equal volume of CFA (0.5 mg ml⁻¹ *M. butyricum*; Difco) or ICFA (Difco) on day 0, followed on day 21 by an intraperitoneal injection of 100 μ g of the same collagen type dissolved in acetic acid. *a*, Onset and incidence of arthritis in male mice given type II collagen in CFA (●); type II collagen in ICFA (○); type I collagen in CFA (◇). *b*, Onset and incidence of arthritis in female mice given type II collagen in CFA (▲); type II collagen in ICFA (△), type I collagen in CFA (□).

examination. Several joints of a single animal were often involved, sometimes so severely that the mouse had to be killed. The onset of arthritis in female mice injected with type II collagen in ICFA was delayed—swelling was never seen before day 36 in any joint. The incidence of the disease was lower in male and female mice injected with type II collagen in ICFA (67%) than in animals receiving type II collagen in CFA (100%).

The incidence and day of onset of arthritis in mice injected in four separate experiments are shown in Fig. 1. Of 40 mice injected with type II collagen in CFA, all developed arthritis, compared with 18 of 27 injected with type II collagen in ICFA. None of the 12 mice injected with type I collagen in CFA, or of the 17 mice injected with acetic acid and CFA, showed signs of the disease (Table 1).

Histological examination of affected joints from animals receiving type II collagen either in CFA or ICFA revealed initial oedema of the synovium and perisynovial tissues, followed by synovial hyperplasia. There was an early massive, predominantly polymorph and subsequent mononuclear cell infiltration into the synovium, sub-synovium, joint cavity and periarticular tissue. This was followed by pannus development, fibrin deposition, and cartilage and bone erosion with new bone formation. Late in the course of the disease the cellular infiltration and oedema were reduced, but by then many joints had become ankylosed, and most recognisable synovium and joint cartilage had been destroyed. The vertebrae were rarely affected, involvement occurring only several weeks after initial peripheral joint swelling. Very occasional, small inflammatory foci in the ear or tail were seen in a few animals.

Several important negative observations were made. There was no clinical or histological evidence of arthritis after injection

of type I bovine collagen or acetic acid in CFA. Uninoculated control animals kept in the same cage as mice injected with type II collagen in CFA did not develop any clinical or postmortem evidence of arthritis. Synovial cavity washes of affected and unaffected joints from 17 DBA/1 mice were cultured for mycoplasma and all gave negative results. There was no significant growth of bacteria when synovial cavity washes from one unaffected and four affected animals were cultured anaerobically and aerobically, and no bacteria were seen in histological preparations of joints.

There are differences between the disease in the rat as described by Trentham *et al.*¹ and that seen in the mouse. Arthritis in the rat appeared earlier and it could be produced by immunisation with antigen in either CFA or ICFA. Mice responded less well to use of ICFA as adjuvant. A single intradermal injection has produced the disease in the rat, but only a schedule of initial intradermal immunisation followed by a subsequent challenge has been used in the mouse. We have found in both species, that involvement of second, third and fourth paws may occur up to 4 weeks after swelling of the first joint and that the number of joints affected varies between animals. The histology in the two species is similar.

Initial studies in other strains of mice treated in the same fashion showed that arthritis may sometimes be produced, but its clinical and histological incidence was variable, and the severity has been less than in the DBA/1 mice: SWR (major histocompatibility type H-2q)—4/15 (total number with clinical or histological lesions/total number treated); SJL (H-2s)—4/6, BALB/c (H-2d)—4/27; BIO.G (H-2q)—4/11; DBA/1 mice from the Jackson Laboratory, Bar Harbour, Maine—2/5 (the Carsholton colony was derived from this colony 25 years ago (M. W. Festing, personal communication); male and female mice from both colonies are histocompatible on skin grafting and *in vitro* mixed lymphocyte culture (L. Berumen, personal communication). Groups of 5–10 mice of the following strains did not develop any joint lesion after treatment with collagen type I or II in CFA or ICFA—C57B1/10ScSn (H-2b), CBA/CA (H-2k), BIO.A (5R) (H-2i5), BIOBR (H-2k) and DBA/2 (H-2d).

It is known that the immune response to the native helical portion of the bovine type II collagen molecule differs in strains of inbred mice³ and that the response appears to be under H-2 linked control⁴. Trentham *et al.*⁵ have shown that in rats the conformational antigenic site on the collagen molecule is important in induction of arthritis, since isolated α -chains of type II collagen did not produce arthritis when injected intradermally in either CFA or ICFA. Initial work has been undertaken here to study the anti-type II collagen antibody response in susceptible and resistant strains of mice, the role of the histocompatibility type and to examine the immunological processes involved.

Table 1 The incidence of arthritis induced by bovine collagen type II in DBA/1 mice

Bovine collagen type given on day 0 and on day 21	Adjuvant given on day 0	No. of mice affected by day 62/total mice given treatment
Male	II CFA	24/24
	— CFA	0/10
	—	0/5*
	II ICFA	12/15
	— ICFA	0/4
Female	I CFA	0/5
	II CFA	16/16
	— CFA	0/7
	II ICFA	6/12
	I CFA	0/7

* Kept in same cage as affected animals.

The finding that an arthritis develops in mice after immunisation with type II collagen lends some support to the hypothesis of Trentham *et al.*¹ that an immune reaction to collagen may be involved in the pathogenesis of arthritis. Use of the mouse disease as a model should avoid the risk of adjuvant arthritis common to experiments in the rat, and it should also permit ready analysis of the role of histocompatibility type and immune response genes.

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Human antibody reacts with a B-cell subset in man to induce B-cell differentiation

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The events leading to B-cell differentiation and maturation into plasma cells in man are still poorly defined. Human B-cell differentiation has mainly been studied after triggering by nonspecific mitogens which react with unknown B-cell-surface structures. T cells and T-cell factors play an important part in this B-cell activation¹. It is therefore interesting that we found that some patients with the rare immunodeficiency disease, the Wiskott-Aldrich (WA) syndrome, possess an IgM antibody which reacts only with a subset of human B-cells from the peripheral blood and tonsils and which is able to induce B cells to differentiate into plasma cells *in vitro*. This triggering is largely independent of T cells and in certain conditions occurs in the absence of detectable B-cell proliferation. In view of certain recent findings in some immunologically defective strains of mice such as CBA/N, genetic immunodeficiencies in man may represent useful models for investigating human B-cell subsets and the process of B-cell activation. We have therefore looked for B-cell antibodies in the serum of WA patients because, in addition to an immune defect of poorly understood mechanism², these patients have major thrombocytopenia and are therefore multi-transfused. We found that the serum of seven of nine WA patients contained an antibody reacting by cytotoxicity and immunofluorescence tests with a subset of both normal human and, unexpectedly, of the patients' own B cells.

A two-step double labelling procedure was used for the immunofluorescence experiments. In the first step, membrane-bound B-cell immunoglobulins (Ig) were redistributed with fluoresceinated IgG or F(ab')₂ fragments from antibodies to human Ig (or μ chains), allowing the enumeration and identification of B cells. In the second step, the cells were incubated in non-capping conditions with heat-inactivated sera of the various patients or controls, washed and finally stained with rhodamine-conjugated F(ab')₂ fragments of monospecific rabbit IgG antibodies to human Ig chains³. Whereas the control sera, including 15 patients with varied immunodeficiencies, did not stain a sizeable population of B cells, the serum of seven WA

patients reacted with 30-60% of B cells from human tonsils and from the peripheral blood of all 30 normal donors tested. The antibody belongs to the IgM class and is polyclonal because the 19S (and not 7S) fraction obtained by preparative ultracentrifugation of the serum in a sucrose density gradient was reactive and, in immunofluorescence tests staining was observed only using F(ab')₂ fragments of antibodies to μ , κ and λ chains (and not to γ , α or δ). Most sera had a titre of 1/4 to 1/16 and reacted with B cells at both 4 °C and 37 °C. Two sera had an end point of 1/32 and 1/128 respectively and were also studied by cytotoxicity at 25 °C on purified B- and T-cell populations from selected donors with various HLA phenotypes. These experiments confirmed that the antibodies were unreactive with T cells and killed a subset of B cells.

The reactive antigenic determinant on the surface of B cells is independent of surface Ig (and specially IgD) as shown by double-labelling and redistribution experiments. Double labelling experiments also showed that the reactive structure is unrelated to Ia-like (or Dr) antigens, as could be expected in view of its presence on only a B-cell subset. For the same reason it seems very unlikely that the WA antibody reacts with other well defined B-cell-surface molecules such as the receptors of IgG, IgM, complement components, or Epstein-Barr virus. In addition it should be recalled that the antibody was unreactive with T cells and monocytes, as well as red cells and platelets.

The antigenic structure is presumably a marker of a unique subset of B cells and does not seem to be a differentiation antigen since we found that it is expressed on pre-B cells of a patient with pre-B-cell acute lymphoblastic leukaemia, on all leukaemic cells from two of five patients with B-derived chronic lymphocytic leukaemia and on the plasma cells from two of four myeloma cases. Experiments to define the biochemical properties and molecular weight of this antigenic structure are in progress. It is probably distinct from the glycoprotein of molecular weight 54,000 (gp 54), recognised by a rabbit antiserum and recently described by Wang *et al.*⁴, which is also a marker of a subset of human B cells, since interaction of these surface molecules with their specific antibodies mediates different functional effects.

Whereas antibodies to the gp 54 antigen stimulated B lymphocytes to proliferate but not to differentiate into plasma cells, we found that the WA antibody does induce B-cell maturation. Table 1 shows that when peripheral blood lymphocytes were cultured in the presence of 1% WA serum (or its 19S fraction at a concentration of 10 μ g ml⁻¹), a 5- to 20-fold increase in the percentage of plasma cells above control cultures was found on day 7, together with a striking cell proliferation and increase in surface Ig-positive cells. The pattern which was

Table 1 *In vitro* induction of B-cell differentiation by WA serum

		Cytoplasmic Ig*				Membrane Ig*			
		μ	γ	α	δ	μ	γ	α	
Expt 1	WA serum	12	8	5	0	23	5	8	
	NHS	1.5	0.5	<0.5	0	7	5	3	
	PWM	15	13	11	0	18	3	9	
Expt 2	WA serum	15	6	3	0	22	2	5	
	NHS	1	0.5	<0.5	0	6	4	2	
	PWM	8	12	11	0	12	2	10	
Expt 3	WA serum	14	6	7	0	21	3	6	
	NHS	0.5	1	0	0	8	4	2	

Mononuclear cells from peripheral blood were isolated by Ficoll-Hypaque gradient. The cells were washed three times in RPMI medium 1640 containing 10% FCS and antibiotics. 5×10^5 cells were incubated for 7 days in a 5% CO₂/95% air incubator in 5 ml of the same medium containing 1% WA serum, 1% normal human serum (NHS) or 50 μ g ml⁻¹ PWM. On day 7, cells were washed and studied for surface and cytoplasmic Ig by direct immunofluorescence with monospecific antisera to human Ig heavy chains.

* Percentage of positive cells.

Table 2 *In vitro* induction of B-cell differentiation by WA serum using B-cell enriched or unseparated PBL and two different batches of FCS

		Unseparated PBL			E-rosette-depleted PBL		
		WA serum	NHS	PWM	WA serum	NHS	PWM
Expt 1	cIg*	27	1.5	21	5	0.5	2.5
Expt 2	cIg*	1	<0.2	8	2	<0.2	<0.2
	SIg*	10	3	15	67	20	34
Expt 3	cIg*	1.5	<0.1	10	3.5	<0.2	0.5
	SIg*	34	7	17	66	18	40

Unseparated peripheral blood lymphocytes (PBL) or B-cell enriched populations obtained by Ficoll-Hypaque gradient separation of lymphocytes rosetted with neuraminidase-treated sheep erythrocytes (two successive separations) were cultured for 7 days with 1% WA serum, 1% normal human serum (NHS) or PWM as described in Table 1. Experiment 1 was performed with the batch of FCS used in Table 1 whereas experiments 2 and 3 were performed with another batch of FCS (see text).

* Percentage of positive cells.

observed was very close to that of pokeweed mitogen (PWM)-induced B-cell differentiation and the isotype distribution of intracytoplasmic Ig showed a slight predominance of IgM over IgG and IgA plasma cells. These figures were obtained with a given batch of fetal calf serum (FCS). When using another batch of FCS, there was still a 5- to 10-fold increase in the percentage of Ig-bearing immunoblasts and of plasma cells in the presence of the WA antibody as compared with normal serum. However, in these conditions, the number of plasma cells in the culture was smaller and the differentiation of B lymphocytes occurred without detectable cell proliferation, as assessed by the absence of thymidine uptake on any day during the period of culture. The discrepancy between these two sets of results using different batches of FCS was explained by the study of the effect of the WA antibodies on purified B cells. When using such purified B-cell preparations, the cell recovery and plasma cell number were similar for both batches of FCS (Table 2). We therefore conclude that B-cell differentiation induced by the WA antibody can occur after depletion of T cells. T cells are needed to obtain B-cell proliferation and optimal differentiation; this T-cell effect requires the presence of a supportive batch of FCS.

Since PWM probably only acts on a given B-cell population¹, experiments were designed to investigate possible relationships between the B-cell subset reacting with the WA antibody and that responsive to PWM. There was no additive effect when lymphocytes were cultured in the presence of both PWM and WA serum. On the other hand, the depletion by cytotoxicity of those B cells reactive with the WA antibody resulted in a 60–80% decrease in the number of plasma cells obtained in PWM-stimulated cultures. These results show that the two B-cell subsets are similar or at least overlapping. Since PWM-induced B-cell differentiation is mediated by T-cell factors, it is worth investigating possible interactions between the WA antibody and a receptor for T-cell helper factors.

These experiments demonstrate that the antibodies to a subset of B cells found in the serum of some patients with the WA syndrome interact with a surface molecule involved in B-cell triggering. To our knowledge, such antibodies have not previously been described in man. Their properties are somewhat reminiscent of those of antibodies directed to the receptor for lipopolysaccharide on mouse B cells⁵ and of anti-Ly 3 antibodies which markedly enhance the development of antigen-specific plaque-forming cells⁶. The surface antigen defined by our antibody may be similar to the murine Ly 3 antigen which is a marker of the B-cell subset absent in CBA/N mice⁶. The availability of the human antibody described here may help to characterise such human lymphocyte markers and to obtain insights into the control of human B-cell activation.

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Osteoclasts derived from haematopoietic stem cells

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The origins of the multinucleated osteoclast have been controversial, with osteogenic precursors¹ and haematopoietic stem cells² as candidates. Recent evidence for the latter is persuasive but circumstantial. We report here direct evidence obtained in radiation chimaeras from a natural cytoplasmic cell marker transmitted by the donated haematopoietic stem cell.

Beige mice (Chediak-Higashi syndrome) have giant lysosomes, present in granular leukocytes and monocytes³ and osteoclasts⁴. Lethally X-ray irradiated (600 rad) osteopetrotic weanling microphthalmic (*mi/mi*) mice have been converted to radiation chimaeras⁵, by intravenous infusion of bone marrow from beige (*bg/bg*) mice. This gives complete regeneration of the lymphomyeloid complex from donor's haematopoietic stem cells, but osteogenic cells and their precursors remain of host-type^{6,7}. In the course of restoration, the osteopetrosis, attributed to a genetic defect of osteoclastic mechanisms, is cured^{8,9}, the process being evident after 2 weeks, advanced at 4 weeks and complete by 6 weeks.

Table 1 shows that the per cent area of giant lysosomal granules in osteoclasts observed electron micrographically increases from a baseline of near zero to 0.15% at 2–4 weeks, a difference statistically significant ($P < 0.001$) but not impressive to a visual observer; at 6 weeks this is nearly 2%, the figure observed for beige mice⁴. This level persists until the last observations, made after 4 months; that is, the osteoclasts of the chimaera, like the granular cells, notably eosinophils, exhibit the characteristics of the beige mouse donor. Osteoblasts remained without giant lysosomes.

Table 1 The per cent area of osteoclasts occupied by giant lysosomes (>1 μm diameter)

Group	Days after transplant (no. in groups)	Mean % of osteoclast area due to giant lysosomes
Experimental	14 (1)	0.18
	28 (3)	0.12
	42 (3)	1.84
	56 (3)	1.87
	120 (2)	1.99 ₅
Control CBA	— (5)	0.02
Control <i>mi/mi</i>	— (5)	0.016
Control <i>bg/bg</i>	— (5)	2.09 $\pm$ 0.58

For each animal, 30–40 photographs of osteoclasts, observed electron micrographically in metaphyses of femora and ilia, were taken at a magnification of $\times 3,000$, two to five pictures being required to cover what seemed to be a single osteoclast. The photographic negatives were analysed on a Quantimet (Cambridge Instruments). The measurements of area in picture points were converted to μm^2 . A computer program was written to coordinate osteoclast area and number of, and area occupied by, giant lysosomes.

We conclude that the ultimate osteoclasts of the chimaera derive from the haematopoietic stem cells of the beige mouse donor. During the first 2–4 weeks, resolution of the osteopetrosis proceeded steadily. Then, the osteoclasts with brush borders observed electron micrographically, although not yet formally enumerated, became more common than in normal mouse bone. However, the emergence of the lysosomal marker in these cells was variable, observable but not impressive. There may be several explanations for this: (1) only a minor population of donor's osteoclasts may be adequate for the job of clearing the redundant bone. (2) Osteoclasts was being pushed to its limit. Osteoclasts working overtime may not form giant lysosomes by fusion of small lysosomes. (3) Osteoclasts of this degree may be a function of uninucleated precursor cells^{10,11} not scored in our investigation. Other elements of the mononuclear phagocyte system, such as tissue phagocytes², have been invoked in osteoclasts, and blood monocytes are reported to be lytic for dead bone^{12,13}. (4) The graft of myeloid tissue confirmed in other investigations^{14,15} by the nuclear marker (T6 chromosome) may have the additional ability to repair the defect of the host's osteoclasts by short-range exchange of 'information', that is, plesiotherapy (πλησιός, near; πάθος, experience) as between lymphocytes and cells of the mononuclear phagocyte system.

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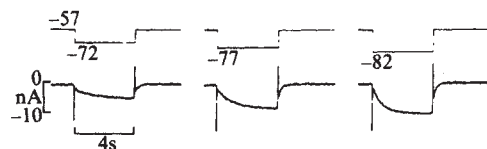


Fig. 1 Voltage clamp records from 7 day HCA in 1.3 mM K_0 . Rise time of voltage control was 5–15 ms. Current records were filtered with a low pass filter having 3 dB cutoff at 50 Hz.

for 7, 12 or 17 days at 37 °C. The tissue was dissociated into its component cells in 0.05% trypsin (1:300 Nutritional Biochemical) by a multiple cycle dissociation process⁷. Cells were allowed to reaggregate in a flask containing tissue culture medium which was placed on a gyratory shaker for 48–72 h. During gyration HCA were formed which were typically from 100 to 300 μ m in diameter (D). The HCA were allowed to adhere to the bottom of a tissue culture dish containing medium 818A (ref. 6). Potassium concentrations (K_0) used ranged between 1.3 and 6 mM. The temperature of the dish was maintained at 37 °C (continuously monitored). Intracellular electrical recordings were made with glass microelectrodes filled with 3M KCl (resistance 15–50 M Ω). Measurements from all three developmental stages were made in the presence of 3 μ M tetrodotoxin (Sigma) which acted to block the fast inward sodium current which seems to be fully developed before day 7 (ref. 8). Voltage-clamp measurements were performed with standard circuitry⁹ on aggregates with $D = 180$ –220 μ m. Current was recorded from the voltage drop across a 10-M Ω feedback resistor of an operational amplifier (Analog Devices, Model 48K) which held the bathing medium at virtual ground via an agar salt bridge and an Ag:AgCl wire. The HCA is suitable for a voltage clamp study of pacemaker currents because: (1) the cells are well-coupled^{10,11}; (2) the specific membrane resistance is large for pacemaker potentials¹²; (3) most of the cells are in the two or three outermost layers of the preparation because of its spherical geometry; and (4) the extracellular cleft spaces are relatively large^{12,13}.

The responses to rectangular voltage clamp steps at 7 days in 1.3 mM K_0 are shown in Fig. 1. These steps produced a time-

Pacemaker currents in chick embryonic heart cells change with development

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The initial heartbeat of the chick embryo occurs shortly after the first day of incubation^{1,2}. The pacemaker of this beat originates in the region of the primitive heart destined to become sinoatrial tissue in the adult³. Individual cells isolated from the atrial and ventricular portions of the embryonic heart are also capable of beating spontaneously at this stage^{4,5}. However, the intrinsic activity of these cells gradually diminishes from about day 7 until day 21 when the chick hatches⁵. We have investigated these changes in automaticity by measuring membrane pacemaker currents with the voltage-clamp technique from aggregates of cells prepared from 7-, 12- and 17-day-old chick embryo cardiac ventricle. We report that there is a voltage and time dependent conductance at 7 days which is markedly reduced by 17 days. The reduction of this pacemaker current parallels the decrease of spontaneous activity in these preparations.

Spheroidal heart cell aggregates (HCA) were prepared using techniques previously described⁶. Briefly, the apical portions of heart ventricles were dissected from chick embryos incubated

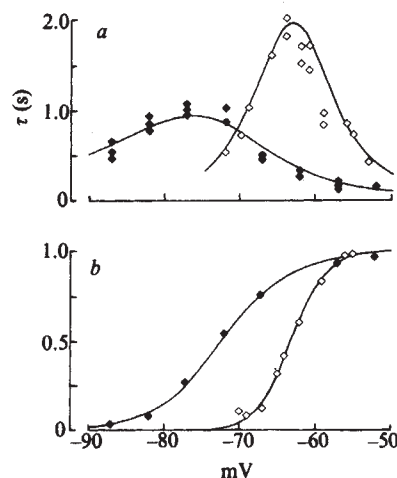


Fig. 2 Analysis of time-dependent currents in 7 (◆) and 12 (◇) day HCA. The currents from all developmental stages could be described by a single exponential function with time constant τ . For each pulse record τ was determined from a photograph or an oscilloscope trace using a Hewlett-Packard 9810A calculator, 9864A digitiser, and 9862A plotter. Solid lines are theoretical best fits to each set of data using modified forms of the model of pacemaker current in cardiac Purkinje fibre of McAllister *et al.*¹⁷. *a*, Voltage dependence of the time constant (τ) obtained from a single 7 or 12 day HCA pooled in each case from 4 different holding potentials. *b*, Normalized current amplitude on return to a single holding potential obtained by the method in ref. 14.

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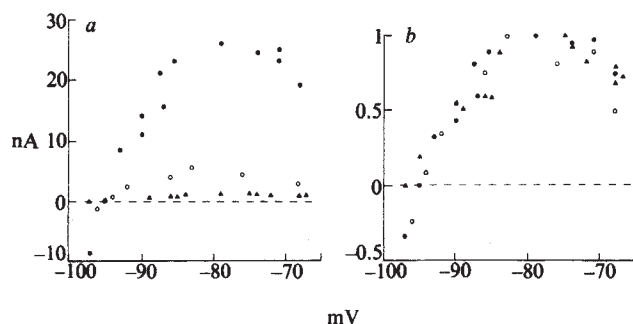


Fig. 3 *a*, Pacemaker channel current-voltage relationship¹⁴ for 7 (●), 12 (○) and 17 day (▲) HCA in 3.5 mM K_o . The points were from a single preparation at each developmental stage. *b*, Normalised channel current-voltage relationships obtained by dividing each set of points in *a* by the corresponding peak current (I_p).

dependent change in membrane current which reached a steady final level corresponding to the voltage to which the membrane was clamped during the step. Successively smaller time-dependent currents were observed at 12 and 17 days, respectively, in the -55 to -120 mV range of membrane potentials. The current changes in this voltage range obeyed first order kinetics amenable to standard Hodgkin-Huxley analysis¹⁴⁻¹⁶ (Fig. 2). The maximum time constant was 1 s at 7 days compared to 2 s at 12 days (Fig. 2*a*), and the range of activation of the kinetics was narrower at 12 days than at 7 days (Fig. 2*b*). The voltage at which the maximum time constant occurred at 12 days appeared to be shifted in the positive direction. The kinetics and the voltage at which the maximum time constant occurred at 17 days were both approximately the same as those at 12 days. Changes of K_o in the 1.3 to 6 mM range did not alter the voltage range or the time scale of the kinetics.

The amplitude of this pacemaker current may be ascribed to ion flow through membrane channels, each of which is gated by a particle having kinetics as described in Fig. 2. The voltage dependence of the channel current may be determined from the ratio of the time-dependent current change during the clamp step to the time-dependent current change after the voltage returns to the holding potential¹⁴. The results of this analysis for the three developmental stages in K_o of 3.5 mM are shown in Fig. 3*a*. The current inwardly rectifies and has a negative slope region positive to -80 mV. The relative voltage dependence of the channel current was nearly the same at all three stages (Fig. 3*b*). The peak channel current (I_p) was approximately independent of K_o , although the voltage at which I_p occurred was shifted by K_o and the reversal potential of the channel current changed with K_o like a potassium electrode. The pooled results for I_p were 27.2 ± 2.6 nA ($\pm$ s.d., $n = 7$) at 7 days, 3.4 ± 1.1 nA ($n = 5$) at 12 days and 0.8 ± 0.4 nA ($n = 5$) at 17 days. The reduction of this current could be produced either by a reduction of the channel density or by a reduction of the single channel conductance.

This study is the first analysis of the changes occurring in the ionic pacemaker currents of heart muscle cells during embryonic development. The contribution of the pacemaker current we have described to the control of beat frequency requires analysis of the time-independent current in the pacemaker voltage range, which also changes during development. This current becomes less inward with development and seems to be the dominant factor responsible for the loss of automaticity in these preparations.

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PGE₂ stimulates gastric chloride transport: possible key to cytoprotection

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Prostaglandins protect the stomach against a variety of noxious agents independently of effects on acid secretion, but the mechanism of this 'cytoprotection' is unknown¹. We recently proposed² that gastric surface cells extrude or eliminate luminal acid by a process analogous to that described in squid axon³, snail neurone⁴, and barnacle muscle⁵. Influxing luminal H^+ combines with HCO_3^- which has entered the cell in exchange for intracellular chloride, probably at the nutrient membrane. Dehydration of the resulting H_2CO_3 into CO_2 and H_2O is catalysed by carbonic anhydrase, which is present in surface cells in large amounts⁶. Interference with this chain of reactions at any point frequently causes ulceration². We have examined the effect of 16,16-dimethylprostaglandin E₂ (PGE) on different segments of this protective mechanism and show here that the protective effects are intimately associated with stimulation of chloride transport. All experiments were done *in vitro* eliminating any effects of prostaglandin on mucosal circulation.

Fundic or antral mucosa of bullfrogs (*Rana catesbeiana*) were mounted in lucite chambers after the frog was pithed and the muscular coat stripped from the mucosa². The flux of $^{36}Cl^-$ was measured from nutrient (N) to secretory (S) solutions ($J_{Cl_{ns}}$), and vice versa ($J_{Cl_{sn}}$) in paired (fundus) and unpaired (antrum) tissues while the potential difference (p.d.), resistance (R) and short-circuit current (I_{sc}) were recorded⁷. Under standard open circuit conditions 10^{-5} M PGE caused a transient decrease followed at 60 min by a significant increase in $J_{Cl_{ns}}$. A concomitant rise in $J_{Cl_{sn}}$ and I_{sc} was observed. The increase in I_{sc} is probably caused by the fact that the inhibition of H^+ secretion is not accompanied by a decrease in the net transport of Cl^- (Fig. 1). Similar increases in unidirectional J_{Cl} were observed in short-circuited antrum except that the initial drop in $J_{Cl_{ns}}$ was not present (Fig. 2). The marked decrease in $J_{Cl_{ns}}$ and $J_{Cl_{sn}}$ noted when HCO_3^- is removed from the nutrient solution in metiamide-inhibited tissues was not influenced by the presence of 10^{-5} M PGE in N ($n = 4$). Addition of 4 mM SITS (4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonic acid) known to block anion transport in a variety of tissues, to N of fundic mucosa for 160 min decreased $J_{Cl_{ns}}$ from 9.49 ± 0.96 to $4.72 \pm 1.03 \mu Eq h^{-1} cm^{-2}$ and $J_{Cl_{sn}}$ from 5.64 ± 1.07 to $3.01 \pm 0.44 \mu Eq h^{-1} cm^{-2}$ (mean $\pm$ s.e.; $n = 3$) and also reduced the p.d. and I_{sc} by 28.2% and 42.6% respectively. Histamine-stimulated H^+ secretion was decreased 49% by SITS. When 10^{-5} M PGE was added to fundic mucosae

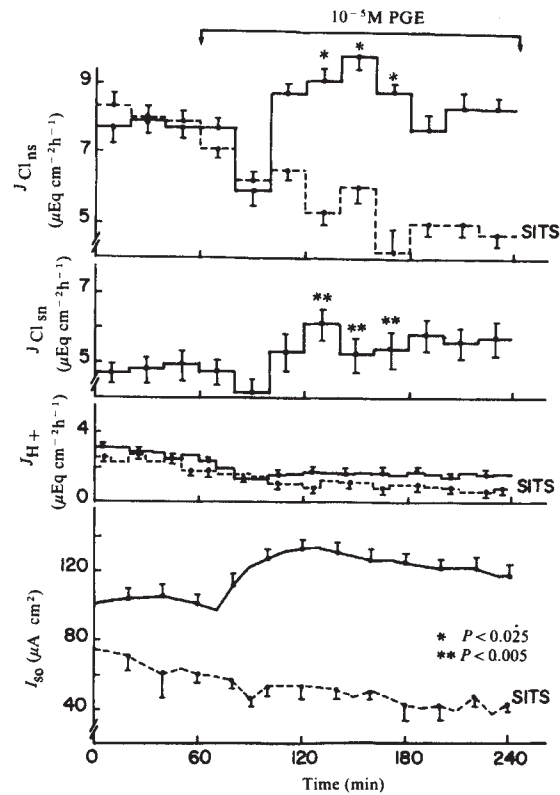


Fig. 1 Effect of 10^{-5} M PGE on Cl^- transport in fundus under open circuit conditions. The bathing solutions contained (in mM) 95 NaCl, 23 Na isethionate on the secretory side and 87 NaCl, 18 NaHCO_3 , 4 KCl, 2 CaCl_2 , 1 MgCl_2 , 1 KM_2PO_4 and 10 mM glucose on the nutrient side and were gassed with 95% O_2 –5% CO_2 . At 60 min after adding PGE, J_{Clns} ($n = 7$) and J_{Clin} ($n = 7$) increased significantly over the baseline values. This did not occur in 7 untreated controls. PGE inhibited H^+ secretion and increased p.d. from 28.6 ± 2.53 to 38.4 ± 1.73 mV (mean $\pm$ s.e.) 60 min after application. R remained stable during this period (205 ± 15.9 to 206 ± 18.52 cm^2). Pretreatment with SITS, 4×10^{-3} M for 60 min led to a decrease in J_{Clns} ($n = 6$) which was not influenced by PGE. Data from baseline and test periods were evaluated by Duncan's test. Comparisons between the means of these two periods were made by the t -test for paired values.

Table 1 Ulceration of gastric sacs					
Buffer	Gas	<i>n</i>	Ulcerated	<i>P</i>	
Control					
5 mM PO_4^{3-}	100% O_2	16	14	0.004	
PG-pretreatment					
5 mM PO_4^{3-}	100% O_2	16	6	NS	
5 mM PO_4^{3-} (Cl^- -free)	100% O_2	16	11		
Control					
SITS + 5 mM PO_4^{3-}	100%	8	8		
SITS + 18 mM HCO_3^-	95% O_2 –5% CO_2	8	4		
PG-pretreatment					
SITS + 18 mM HCO_3^-	95% O_2 –5% CO_2	8	4		
SITS + 5 mM PO_4^{3-}	100% O_2	8	7		

Tied sacs of inverted gastric mucosa (mucosa inside) were incubated in different nutrient buffers circulated by the indicated gas. After 1 ml of artificial gastric juice, containing 40 mg % pepsin and 20 mM HCl, had been instilled into the sac lumen, the tissue was incubated for 4 hours. PG-pretreatment means addition of 3×10^{-6} M PGE 60 min before instillation of artificial gastric juice. The Fisher's exact test was used for statistical evaluation. NS, not significant.

pretreated with SITS, 4 mM no effect on J_{Clns} was observed (Fig. 1).

Forte⁷ has described three components of J_{Clns} in amphibian gastric mucosa. In addition to the component of active transport dependent on the presence of O_2 and to a small diffusional component, a major fraction of J_{Clns} is represented by exchange diffusion dependent on the presence of Cl^- in the secretory solution. Since we postulate that the availability and exchange of cellular Cl^- for nutrient HCO_3^- are vital for protection of surface cells against luminal H^+ , we tested the effect of PGE on exchange diffusion according to Forte⁷. When Cl^- was removed from the secretory solution, PGE was unable to maintain a normal exchange diffusion of Cl^- from N to S (Fig. 3), indicating that the increased Cl^- fluxes across the tissues induced by PGE are more likely a result of increases in exchange of HCO_3^- for Cl^- , than a direct stimulation of the Cl^- pump.

Prostaglandins stimulate an adenylate cyclase in surface cells but not the histamine-stimulated cyclase of oxyntic cells⁸. We added 10^{-3} M N^2 - O^6 -dibutyryl cyclic AMP to N, of short-circuited antral mucosa and found that J_{Clns} increased markedly within 40 min (Fig. 2).

To test the relationship of these findings to the known cytoprotective effects of PGE, we studied tied sacs of fundic mucosa of the same species known to ulcerate almost invariably in the absence of nutrient HCO_3^- (refs 2, 9). Ulceration was prevented to a highly significant degree by 3×10^{-6} M PGE, in the absence of nutrient HCO_3^- but not when Cl^- was replaced by isethionate and SO_4^{2-} in the bathing solution (Table 1). In sacs pretreated with 4 mM SITS, PGE failed to prevent ulceration not only in the absence of nutrient HCO_3^- but even when 18 mM HCO_3^- was present in the nutrient solution (Table 1). The protection afforded by the presence of HCO_3^- in tissues exposed to SITS was not enhanced by PGE.

We conclude that PGE stimulates J_{Clns} and J_{Clin} in a process possibly involving cyclic AMP. The failure of PGE to preserve forward Cl^- flux in the absence of Cl^- in the secretory solution suggests that PGE stimulates the exchange of cellular Cl^- for nutrient HCO_3^- , a vital step in the protection of the surface cell against the hostile environment of luminal acid. The importance of the availability of nutrient HCO_3^- for such an exchange is emphasised by recent studies (in preparation) showing that

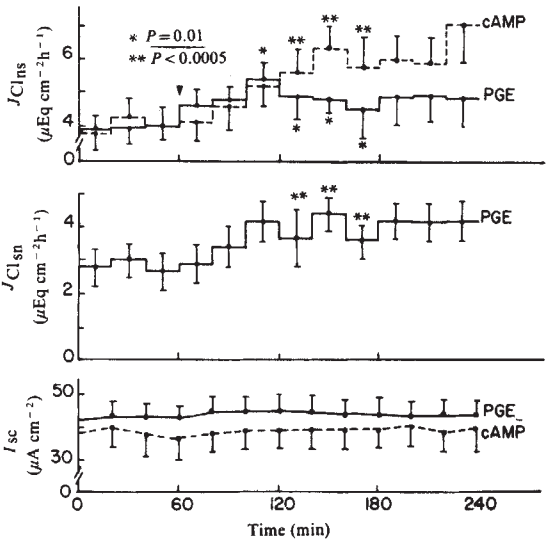


Fig. 2 Effect of 10^{-5} M PGE on Cl^- transport in antrum under short-circuit conditions. J_{Clns} ($n = 8$) and J_{Clin} ($n = 6$) increased significantly over the baseline period values. This did not occur in 8 untreated controls. Cyclic AMP 10^{-3} M also increased J_{Clns} . Arrow indicates addition of drug to the nutrient solution. Data evaluated as for Fig. 1.

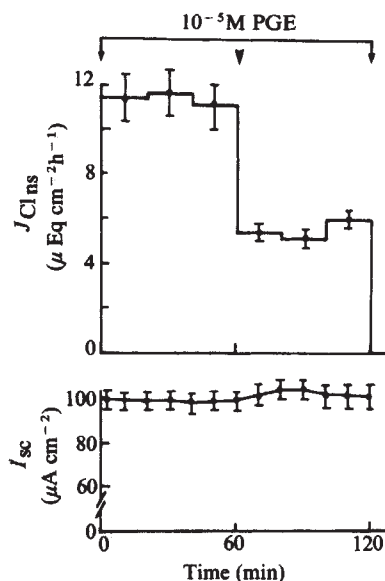


Fig. 3 Effect of 10^{-5} M PGE on the exchange diffusion component of $J_{C_{lin}}$. At the arrow the secretory solution was replaced with 118 mM Na isethionate and 2 mM K_2SO_4 . Duncan's test was used for statistical evaluation.

protection of the mucosa can be achieved with either a high concentration of nutrient HCO_3^- or by the actively stimulated oxyntic cell and its consequent alkaline tide. The protective mechanisms of the gastric epithelial surface cell bear many analogies to the acid extruding pump demonstrated in other tissues³⁻⁵.

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Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone

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Cholinergic excitation of vertebrate neurones is frequently mediated through the action of acetylcholine on muscarinic (atropine-sensitive) receptors. This type of excitation differs substantially from the better known nicotinic excitation. One difference is that, instead of an increased membrane conductance, a decreased conductance (to K^+ ions) frequently accompanies muscarinic depolarisation. This has been detected in sympathetic¹⁻⁴, cortical⁵ and hippocampal⁶ neurones. Using voltage-clamped frog sympathetic neurones we have now identified a distinctive voltage-sensitive K^+ -current, separate from the delayed rectifier current, as the prime target for muscarinic agonists. We have termed this current the M-current, I_M .

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For our experiments we used isolated lumbar sympathetic ganglia from bullfrogs (*Rana catesbeiana*), from which the connective tissue was removed with trypsin (1% for 10-15 min): this treatment did not materially affect the currents under study. The ganglia were perfused with Ringer's solution at 22 °C containing 2.5 mM KCl, 2 mM $CaCl_2$, 2 mM Tris (buffered to pH 7.2 with HCl), and (usually) 10 mM $MgCl_2$ to improve electrode sealing. Individual cells were impaled with two microelectrodes filled with 3M KCl (resistances 20-50 MΩ), one for recording membrane potential and one for passing or recording current.

Some general properties of the M-current are illustrated in Fig. 1. This cell was held under voltage-clamp at a relatively depolarised membrane potential (-30 mV), at which there is normally a substantial and steady outward M-current. On applying a hyperpolarising step-command, the channels carrying the M-current gradually close, giving rise to a slow inward current relaxation after the initial fast ohmic step. Channel closure is indicated by the fact that the membrane conductance falls during this relaxation, the ohmic step at the end of the command being smaller than that at the beginning. Repolarisation then produces a slow outward relaxation as the M-channels open again. (On repolarising from membrane potentials more negative than -70 mV the currents are complicated by the appearance of another, more transient outward current analogous to the rapidly inactivating A-current reported in molluscan neurones^{7,8}.)

With increasing step-commands the inward relaxation reversed in direction at command potentials between -70 and -100 mV (-74 mV in Fig. 1). This reversal potential shifted in the depolarising direction by 20-25 mV on raising $[K^+]$ out from 2.5 to 8 mM, and by 40-55 mV at 25 mM. Thus, I_M is largely, but perhaps not exclusively, a K^+ current.

The M-channels seem to be fully closed at membrane potentials more negative than -60 mV, as hyperpolarising commands from such potentials yielded only ohmic current steps. Above -60 mV the conductance attributable to the M-channels showed a sigmoidal increase, maximising at -10 to 20 mV (Fig. 1c). The position and total amplitude of the conductance-activation curve did not vary using different holding potentials between -20 and -50 mV, indicating that I_M does not show time-dependent inactivation within its activation range. The slope of the mean activation curves corresponded to an 8-fold shift in the equilibrium between open and closed M-channels for 6-7 mV depolarisation, as though the state of the channels was controlled by a tetravalent voltage-sensing particle moving through the entire membrane potential⁹. Both inward and outward M-current relaxations showed an approximately exponential time course (see Fig. 3), whose time constant diminished with hyperpolarisation from a maximal value of 150-200 ms at between -40 and -30 mV. These kinetic properties distinguish the M-current very clearly from the conventional delayed rectifier current⁹. Thus, in our bullfrog neurones, the activation threshold for the latter was about -25 to -20 mV, while the time constants for the repolarising tail-currents of the delayed rectifier were an order of magnitude faster than those for the M-current at comparable membrane potentials (see Fig. 4).

To test the effect of muscarinic receptor activation on the clamp currents we used ($\pm$)muscarine as the preferred agonist; this has minimal nicotinic activity on ganglion cells¹⁰, thus obviating the need for nicotinic blockers. Under current-clamp (Fig. 2a), muscarine produced the characteristic slow depolarisation and conductance decrease previously described for other muscarinic agonists^{1,4}. (In many cells muscarine also induced prolonged repetitive spike discharges following injection of current pulses.) Under voltage-clamp (Fig. 2b) the depolarisation was replaced by a steady inward current at depolarised membrane potentials, while the reduced conductance was manifest as a reduced current response to a hyperpolarising step-command.

Both the steady inward current and the reduced steady-state

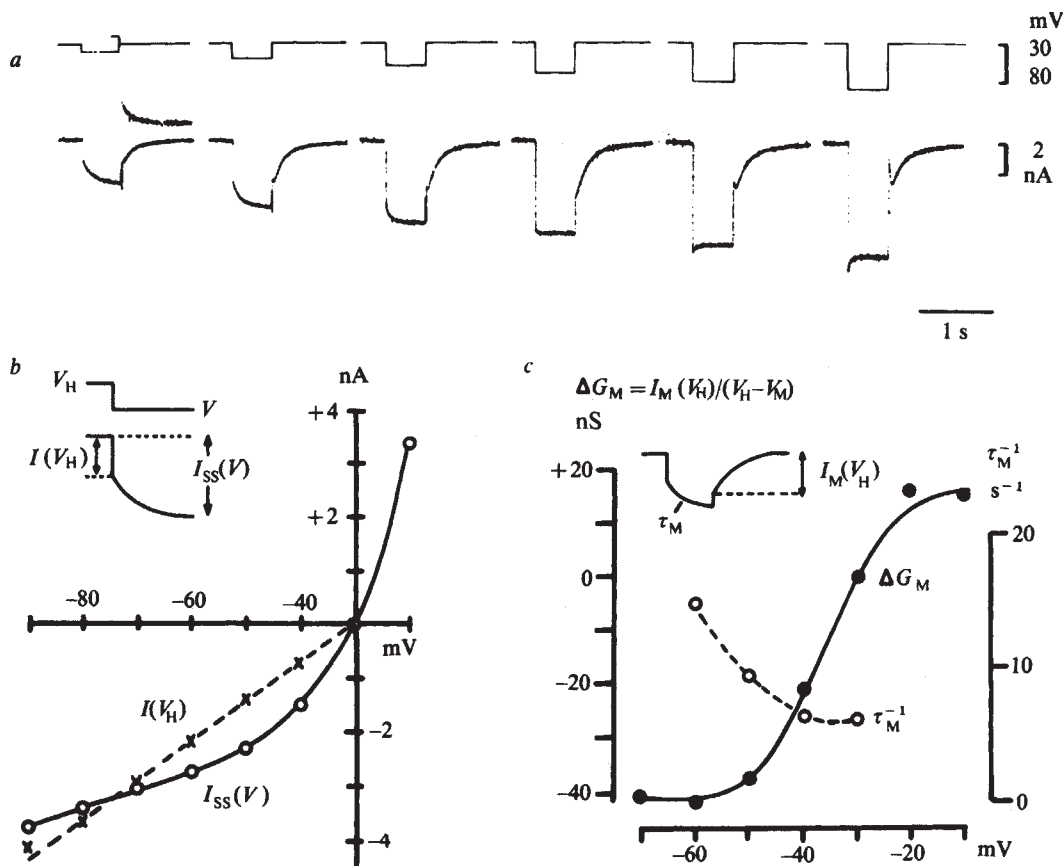


Fig. 1 M-currents in a voltage-clamped bullfrog neurone. The records in *a* show clamp-currents (bottom trace, inward current downwards) recorded during hyperpolarising step-commands from a holding potential (V_H) of -30 mV to command potentials (V) of -40 to -90 mV. The inward current in the first panel is the slow tail of repolarising current after returning to V_H from a depolarising step-command to -20 mV. (This current record is shifted upwards for clarity; the outward current during the command is off-scale). In *b* the instantaneous ($\times$) and steady-state ($\circ$) currents recorded in *a* are plotted against command potential (V). The instantaneous current is the amplitude of the fast transient $I(V_H)$ and the steady-state current is the total current measured after the slow relaxation is complete, $I_{ss}(V)$ (see diagram above). The intersection of the two gives the reversal potential (V_M) for the slow relaxation. The curves in *c* show the change of conductance attributable to closing and opening of the M-channels (ΔG_M , $\bullet$) and the reciprocal of the time constant of the slow relaxation (τ_M^{-1} , $\circ$) plotted against

command potential V . ΔG_M was calculated from the amplitudes of the M-current tails observed on repolarising from V to V_H (-30 mV), divided by the driving force $V_H - V_M$. (At $V = -20$, -10 mV only the slow component of the tail was measured, see Fig. 4b). τ_M was calculated from semi-logarithmic plots of the slow inward relaxations on hyperpolarising from V_H to V (see Fig. 3 for examples). τ_M At -30 mV is the mean value for the relaxation observed on repolarising from V to $V_H = -30$, τ_M for inward and outward relaxation at the same final potential being equal.

conductance produced by muscarine result almost exclusively from a reduction of the M-current. Thus, since the M-channels would be closed at the end of the hyperpolarising command in Figure 2b ($V = -60$ mV, see Fig. 1c), the amplitude of the outward relaxation on repolarising the cell to -30 mV provides a measure of I_M at the holding potential of -30 mV. This repolarising M-current was depressed by an amount (2.2 nA) precisely equal to the magnitude of the steady inward current. Furthermore, muscarine produced negligible inward current at

-60 mV. Direct proportionality between net inward current and I_M reduction throughout the potential range for I_M activation was confirmed in other experiments using different holding and command potentials. Finally, it may also be noted in Fig. 3 that the membrane conductance measured from the amplitude of the ohmic currents was greatly reduced at -30 mV but hardly at all at -60 mV.

From the time course of the M-current relaxations (Fig. 3) it appears that muscarine simply depressed the current amplitude

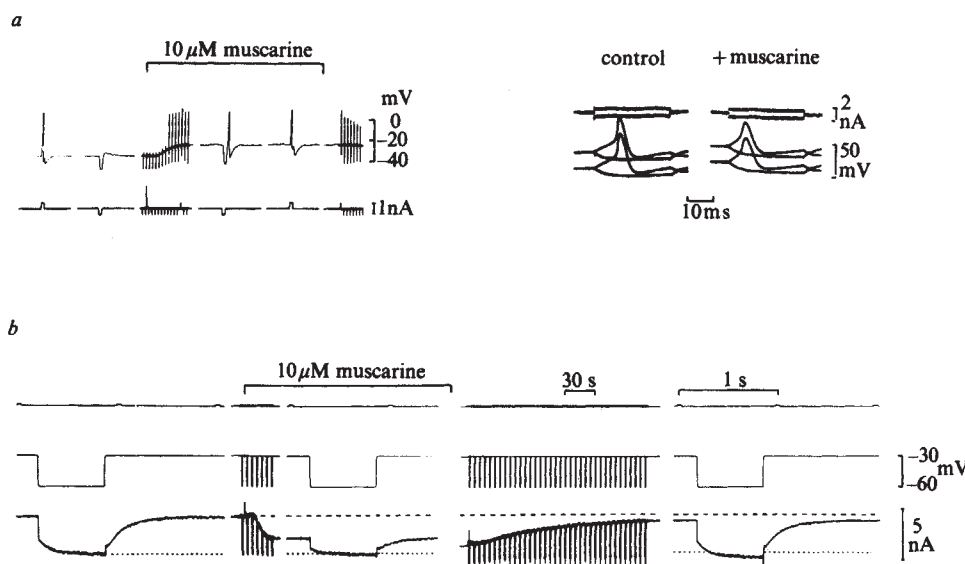


Fig. 2 Effects of muscarine on a single neurone recorded under: *a*, current-clamp, and *b*, voltage-clamp. The three traces on the chart-strip records read (from above downwards) time in s, membrane voltage, and current. The recorder was periodically accelerated to display the voltage and current transients. Muscarine was applied by rapid bath-perfusion. The oscilloscope records on the right side of the voltage response in *a* show superimposed spikes and electrotonic potentials evoked by depolarising and hyperpolarising current pulses respectively. The current pulses are on the top beam. Two voltage records are shown, the upper one being that recorded through the current-passing electrode via an active bridge circuit. In the voltage-clamp record (*b*, recorded earlier in the same cell), the membrane potential was held at -30 mV and commanded to -60 mV for 700 ms at 5-s intervals, and the induced currents recorded.

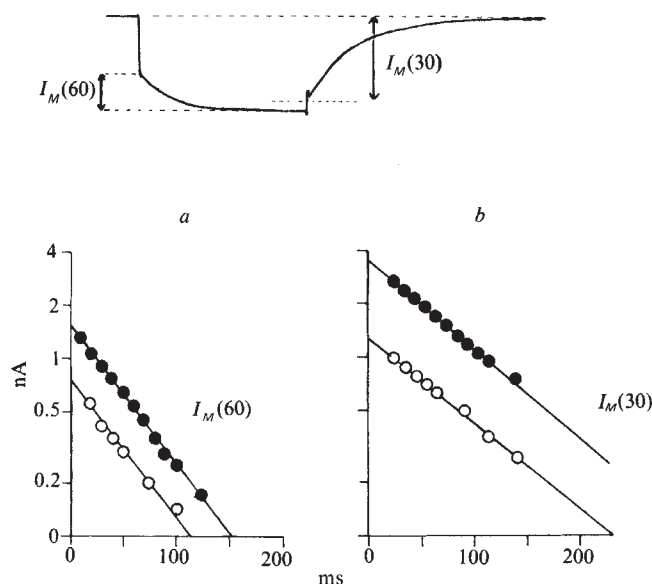


Fig. 3 Semi-logarithmic plots of the slow relaxations observed in Fig. 2b before (●) and during (○) muscarine application. *a*, Inward relaxation on hyperpolarising to -60 mV, $I_M(60)$. *b*, Outward relaxation on repolarising to -30 mV, $I_M(30)$. The diagram above is a tracing of the first control current-relaxation in Fig. 2b, showing the measured currents.

without modifying its voltage sensitivity. Thus the ratio $\tau_M(30)/\tau_M(60)$ remained the same (1.5) in the presence and absence of muscarine. Hence any voltage sensitivity of muscarinic activation^{3,11} appears to reside in the voltage dependence of the resting currents rather than in the action of muscarine *per se*. The effect of muscarine was concentration dependent, half the channels being shut at $1-3$ μM , and was fully antagonised by 1 μM atropine. Varying $[\text{Ca}^{2+}]_{\text{out}}$ between 2 and 8 mM or $[\text{Mg}^{2+}]_{\text{out}}$ between 0 and 10 mM made no qualitative difference to the action of muscarine.

The unique action of muscarine on the M-current is best emphasised by comparing it with tetraethylammonium (Fig. 4): the latter profoundly depresses the delayed rectifier with little effect on the M-current, whereas muscarine suppresses I_M without depressing the delayed rectifier. (We did not see potentiation of muscarine-induced currents in 10 mM TEA solution; compare ref. 12.) The M-current was also unaffected by 1 mM Ni^{2+} which blocks inward Ca^{2+} currents in these neurones (data not shown here), or by $1-5$ mM 4-aminopyridine, which inhibits the A-current of invertebrate neurones⁸.

The function of the M-current is not yet clear. However, it does show some resemblance to the outward ' i_{slow} ' of frog sinus tissue¹³, and may likewise serve to limit firing frequency, by reducing the rate of depolarisation near the threshold for spike generation in the face of a steady or phasic inward current. The increased tendency toward repetitive spike discharges in cells treated with muscarine would accord with this: indeed, this, rather than the depolarisation itself may be the more significant feature of muscarinic excitation.

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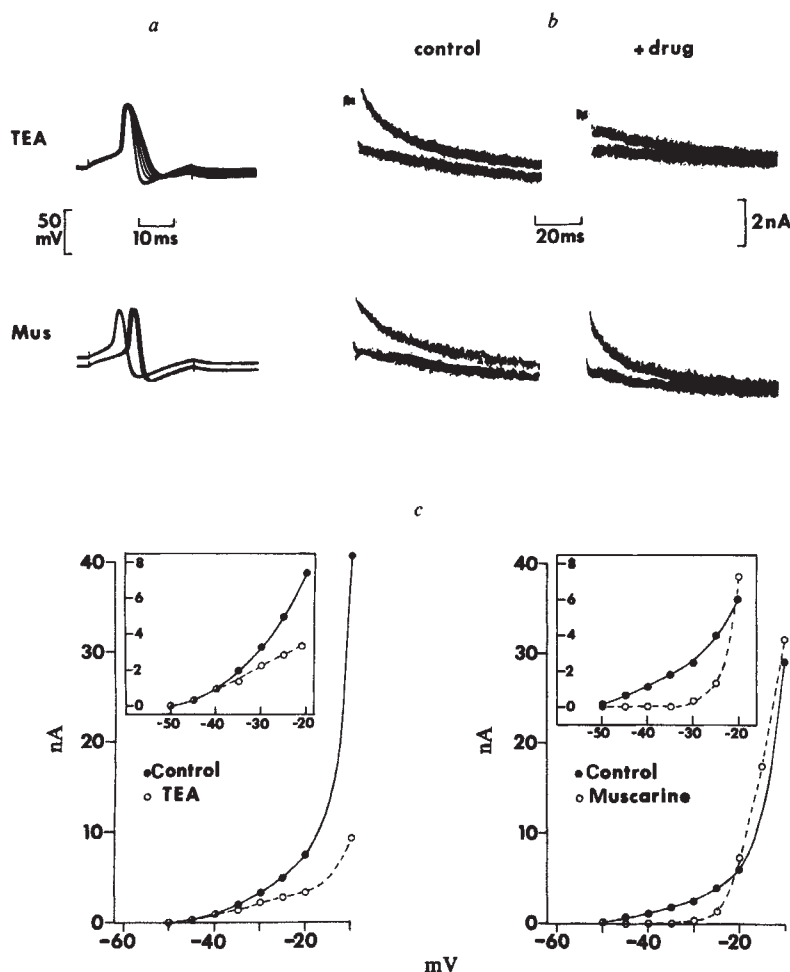


Fig. 4 Comparison of the actions of tetraethylammonium (TEA, 10 mM) and muscarine (Mus, 10 μM) on *a*, unclamped action potentials, and *b* and *c*, repolarising current-tails and steady-state current-voltage curves respectively seen under voltage-clamp. The oscilloscope records in *a* show the action potentials evoked by injecting depolarising current through a single, bridge-balanced recording microelectrode, with control and drug-treated spikes superimposed. TEA prolonged spike-repolarisation without altering the membrane potential, whereas muscarine depolarised the cell without greatly affecting the spike. The records in *b* show the tail-currents seen on repolarising to the holding potential of -50 mV after depolarising commands to either -30 mV (lower trace) or -20 mV (upper trace). In the latter case, the tails showed two components, fast decaying and slow decaying, attributable to the closure of the delayed rectifier and M-channels respectively. TEA (upper records) suppressed the delayed rectifier tails; muscarine (lower records) did not affect the fast, delayed rectifier tail but reduced the amplitude of the slower M-current tail. (The apparent acceleration of the fast tail in muscarine solution is due to the loss of the slow tail.) The graphs in *c* show the steady state current-voltage curves from a single cell exposed first to TEA and then, after washing, to muscarine. The voltage-scale shows the pulse-potential from a holding potential of -50 mV. Current values (ordinates) are the steady currents attained at each pulse-potential minus the leak currents, extrapolated from the ohmic currents following step hyperpolarisations to -60 and -70 mV. The inset curves show the currents up to -20 mV on a larger scale. Note that TEA profoundly depressed the currents at membrane potentials more positive than -30 mV, whereas muscarine selectively depressed the currents below -20 mV.

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Somatostatin production by rat cerebral neurones in dissociated cell culture

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Somatostatin (somatotropin release inhibiting factor, SRIF) is the 14-amino acid polypeptide found to be the active growth hormone-release inhibiting factor in extracts of sheep hypothalamus^{1,2}. Although found in highest concentrations in the hypothalamus, it is widely distributed in the gastrointestinal tract and extra-hypothalamic nervous system. In fact, the cerebral cortex contains a larger total amount of somatostatin than any other brain region or visceral organ³. The source of somatostatin in extra-hypothalamic brain has been unclear. Initially, the hypothalamus was considered the only source, but elegant immunohistochemical demonstrations of positively staining neuronal perikarya in amygdala, hippocampus and neocortex suggested that these areas might be independent sources of somatostatin⁴. In addition, bilateral anterior hypothalamic periventricular lesions reduced somatostatin content in the median eminence to less than 10% of control levels, but did not affect its concentration in the preoptic area, amygdala or cortex⁵. Our laboratory has previously shown that dissociated rat cortical neurones in culture exhibit structural and electrophysiological properties similar to those of adult neurones *in situ*⁶. In conjunction with studies being carried out on the physiological effects of somatostatin on these neurones, we investigated the occurrence of endogenous IR-somatostatin. We show here that dissociated cell cultures from rat cerebral hemispheres in the absence of any hypothalamic tissue produce immunoreactive (IR)-somatostatin. This IR-somatostatin is demonstrable histochemically in an exclusively neuronal population within the cultures.

Tissue was obtained by sterile removal of embryos from pregnant CD rats 15 d post-conception. The embryo skull was removed and slices were taken from antero-lateral cerebral cortex superficial to the lateral ventricles. The deeper portions of the cerebral hemisphere, including areas later giving rise to striatal structures, were discarded, as were the thalamus, hypothalamus, brainstem and cerebellum. The tissue was then minced, digested in trypsin, triturated, filtered for cell clumps, plated and grown in a medium consisting of Eagle's minimal essential medium, 5% heat-inactivated rat serum, 100 mg % glucose, 2 mM glutamine, 20 U ml⁻¹ penicillin and streptomycin. The medium was changed three times per week and cells kept in a 5% CO₂ incubator at 36-37°C. The details of the culturing procedures were as described previously⁶, except that dishes in the present study were coated with collagen and

polylysine instead of a layer of non-neuronal cells. Approximately 250,000 cells were initially plated in each culture dish.

For somatostatin assay, the medium (approximately 1.2 ml) was removed from the culture 3 d after the previous medium change, added to 1 ml 1 M acetic acid and frozen. The remaining cells were rinsed with physiological saline and extracted into 1.5 ml 1 M acetic acid and frozen. At the time of assay, samples were heated in boiling water, sonicated, centrifuged, lyophilised and resuspended in phosphate buffer (0.05 M with 0.1% bovine serum albumin (BSA), 0.1% sodium azide, pH 7.4). Radioimmunoassay was carried out with a well-characterised assay system³.

No IR-somatostatin was detected in the initial cell suspension, in control media samples or in early cultures, but was detected in both cell extracts and media by day 7 of incubation (Fig. 1). Between 7 and 14 d the content in the cellular fraction increased dramatically. Concentrations of IR-somatostatin increased steadily to a maximum at approximately 3 weeks when the cellular fraction contained an average 3,033 ± 7.5 pg per dish and the media contained 326 ± 75.8 pg per ml media. The slightly decreased levels at points around 4 weeks in culture were thought to correspond to declining neuronal survival.

Chromatographic characteristics of the IR-somatostatin of both the cell extracts and the media were determined using a Biogel P-4 column with an ammonium acetate buffer (pH 4.7). The IR-somatostatin from both the cell extracts and the media eluted identically with the synthetic cyclic somatostatin standard. In addition, 8% of the IR-somatostatin in the cell extract migrated more slowly in a separate peak corresponding to a larger form of IR-somatostatin.

Immunofluorescent staining was carried out on cells cultured for 17-33 d on collagen-polylysine-covered glass coverslips. The coverslips were rinsed in a phosphate-buffered saline (PBS) and fixed in 3% formaldehyde in PBS and finally in cold acetone. The acetone step was added to increase the permeability of the fixed cell membranes and resulted in a marked increase in specific immunofluorescence over that obtained with formaldehyde alone, but with preservation of cellular morphology.

After fixation, a rabbit antibody prepared against BSA-conjugated somatostatin was applied at a 1:200 dilution with

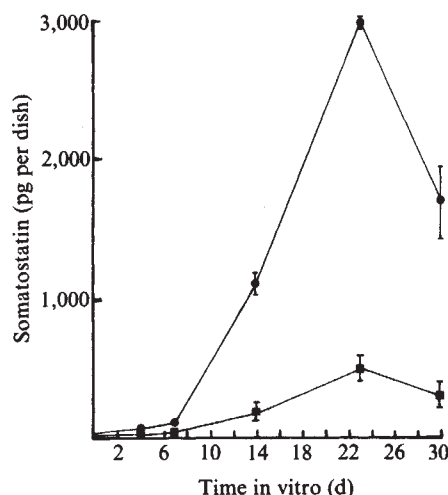


Fig. 1 Graph of IR-somatostatin in cell fraction (●) and in media fraction (■) per 35-mm dish containing dissociated cell culture of rat embryo cerebrum against days *in vitro* (mean ± 1 s.d. for determinations on three cultures). Total volume of cells is very small (<0.1 cm³ per dish) compared with the volume of media (~1.2 cm³ per dish), which emphasises the greater amount concentrated in cells. Levels of IR-somatostatin are undetectable until approximately day 7. Between days 7 and 23 the levels increase dramatically. The late fall-off probably indicates some deterioration in the cultures rather than a specific decline in somatostatin production.

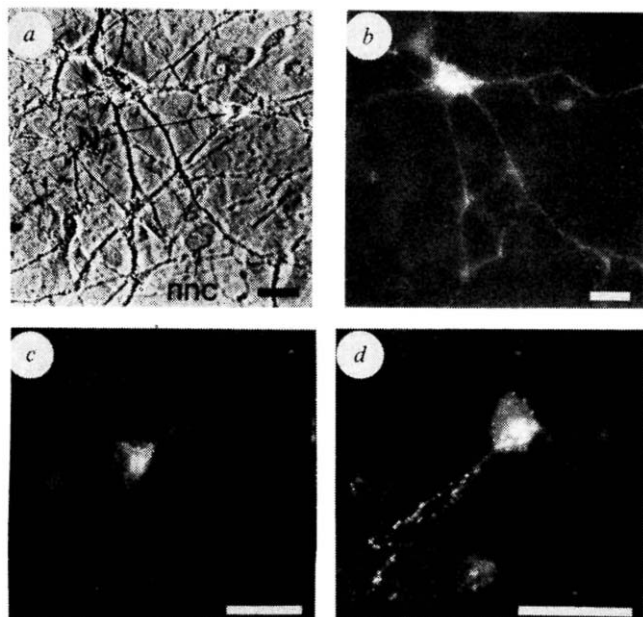


Fig. 2 Phase-contrast and indirect immunofluorescent microscopy of neurons in the dissociated cell cultures. *a*, Dispersed cerebral cell culture at 32 d seen under phase-contrast microscopy. Note flat non-neuronal cells (nnc) as well as neurone cell bodies (N) and neuronal processes. *b*, *c*, *d*, Fluorescence photomicrographs showing cultures stained with anti-somatostatin using indirect immunofluorescent technique. *b*, Same field as *a*, showing positive staining of the perikaryon and its neuronal processes. Note the selective staining of this neurone as distinguished from the several unstained neurone cell bodies and many unstained neuronal processes, prominently seen in *a* by phase contrast. *c*, Pyramidal-shaped neurone positively stained by anti-somatostatin in the presence of more dimly seen negatively staining neurones of roughly the same size in culture at 18 d. Staining in perikaryon of neurones often seemed to be continuous with staining in the proximal part of cell processes. *d*, Neurone positively stained by anti-somatostatin with smaller collections of staining trailing out a neuronal process in an 18-d culture. Scale bars, 25 μ m.

incubation at 37°C for 1 h. Next, fluorescein-conjugated goat anti-rabbit IgG (Meloy Laboratories) was applied at a 1:35 dilution with incubation at 37°C for 1 h. Coverslips were mounted in glycerol at pH 8.5, and viewed and photographed under a Zeiss fluorescence and phase-contrast microscope. Specificity was demonstrated by absence of specific staining when the rabbit anti-somatostatin at 1:100 dilution was adsorbed with an equal volume of 10^{-3} M somatostatin before use. Staining was unaffected by adsorption with gonadotropin releasing hormone, vasopressin or ACTH.

Dichter has previously reported that by using a variety of confirmatory techniques (including silver staining and intracellular recordings), neurones can be recognised in the cultures by their appearance under phase-contrast⁶. All the cells shown by the indirect immunofluorescent technique to contain IR-somatostatin in these cultures were morphologically indistinguishable from neurones identified by these other criteria.

An estimated 3–5% of the neuronal population stained unequivocally in each culture. Most somatostatin-positive cells had two or more large dendritic processes, but by phase-contrast microscopy these positively staining cells could not be distinguished from non-somatostatin-containing neurones. Within cell bodies, IR-somatostatin was predominantly located in a perinuclear distribution which often seemed continuous with staining in the proximal portions of cell processes (see Fig. 2). In axonal processes the staining was most frequently seen in varicosities, and a densely stained collection was often seen at the most distal portion of the process.

These results suggest that neurones of the mammalian cerebral hemispheres can produce somatostatin completely independently of the hypothalamus. The finding of a larger form of IR-somatostatin corresponds with similar findings in many other nervous and gastrointestinal tissue homogenates, including extra-hypothalamic brain^{3,7}. The presence of this larger form in the cellular fraction, and not in the media, suggests a pro-hormone which is not secreted as such, but pulse-chase studies must be made before the presence of such a precursor can be confirmed.

Many studies have reported neurophysiological effects of somatostatin; it depresses the firing of neurones in hypothalamus, cerebellar cortex and cerebral cortex⁸, and in brain-stem, reticular formation and dorsal hippocampus⁹. By contrast, somatostatin has been shown to excite cortical neurones in conscious rabbits¹⁰ and in a rat hippocampal slice preparation¹¹. Its release by calcium-dependent depolarisation has been demonstrated in several neural structures, including hypothalamus^{12,13}, neurohypophysis¹⁴ and dorsal root ganglia¹⁵. Our findings support the view that somatostatin may have a role as an extra-hypothalamic central nervous system neurotransmitter or neuromodulator.

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Cholesterol levels inversely reflect the thermal sensitivity of mammalian cells in culture

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Cholesterol is a primary component of the mammalian cell plasma membrane. Although its function is unknown, it may be of major importance in maintaining membrane fluidity and rigidity¹. In artificial membrane systems, the addition of cholesterol results in a condensing effect—thickening the bilayer and inducing higher order in the acyl chains of the phospholipids². Permeability profiles indicate that the addition of cholesterol into egg-lecithin bilayers increases the half-time of solute transport³. In addition, decreased amounts of sterol in the membrane increase glucose permeability, and, in L cells, increase the transport of rubidium^{4,5}. These studies suggest a role for cholesterol in changing the physical characteristics of the membrane resulting in the alteration of membrane permeability. We now provide evidence that cholesterol may act, presumably via changes in physical membrane properties, with yet another biological consequence; regulating the survival sensitivity of mammalian cells to hyperthermic temperatures.

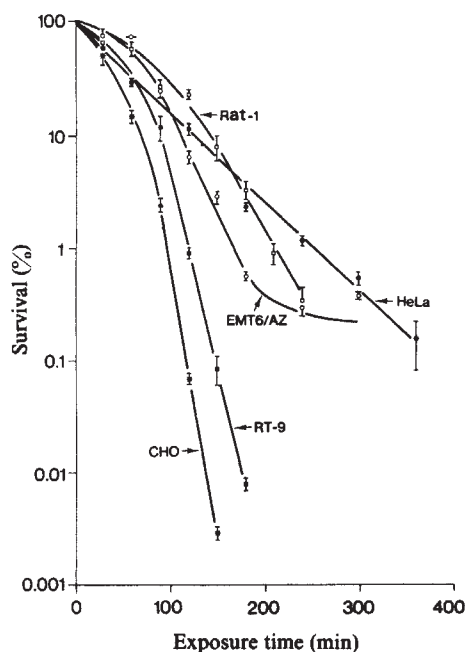


Fig. 1 Thermal survival response of exponentially growing cells in culture. (●), HeLa; (○), EMT6/AZ; (■), RT-9; (□), Rat-1; (×), CHO. After exposure of the cells to 43 °C for the indicated times, cells were collected and plated for colony formation. Standard errors of survival determinations are shown.

Elevated temperatures are known to result in the modification of physical membrane properties in prokaryotic cells, principally by alteration of the amounts of saturated and unsaturated fatty acids⁶. Yatvin⁷ has shown that dietary manipulation of the proportion of saturated and unsaturated fatty acids in *Escherichia coli* membranes acts to modify the thermal sensitivity of these organisms. Prokaryotic membranes contain essentially no sterol and the membrane composition in general is quite different from eukaryotes⁸. However, the physical changes of the prokaryotic membrane during the acclimation to elevated temperatures are remarkably similar to the changes in the mammalian cell membrane when cholesterol is introduced. In both cases, the membranes become more ordered and less permeable.

We have previously shown that one immediate response to exposure to elevated temperatures is an increase in plasma membrane permeability⁹. Recently, we have demonstrated that modification of membrane permeability can also lead to modification of cellular viability at hyperthermic temperatures¹⁰.

Guided by these observations, we added whether the known different heat sensitivities of various mammalian cell lines in culture¹¹ would reflect differences in the amount of cholesterol in the unheated cells. One would expect that elevated levels of cholesterol in untreated cells might be expressed as a higher survival rate following hyperthermia treatments. Hyperthermia in this study represents an elevation of temperature from 37 °C to 43 °C.

Cultures of HeLa, EMT6/AZ, Rat-1, RT-9, and CHO cell lines (see Table 1 for identification of cells lines) were maintained and treated in log phase growth in McCoy's 5A medium supplemented with 20% fetal calf serum, 100 units ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin (all from GIBCO). All lines were grown in a 5% CO₂-95% air atmosphere at 37 °C. Doubling times, under these conditions for the HeLa, EMT6/AZ, Rat-1, RT-9, and CHO cells, were 20–22, 15–16, 15–17, 14–15, and 13–15 h, respectively. Colony-forming efficiencies for untreated cells growing at 37 °C, for these same lines were 60–80%, 60–80%, 50–70%, 60–80%, and 80–95% respectively.

Cell survival, determined by the colony-forming ability of single cells, was used as the index of the thermal sensitivity of the various cell lines studied. The procedure we used, and the hyperthermic treatment conditions in our temperature-controlled water bath (temperature uniform and constant to ± 0.1 °C), have previously been described in detail^{12,13}.

For cholesterol determination, 2.5×10^7 cells per sample were collected by scraping with a 'rubber policeman', washed in Puck's saline A (GIBCO), and sonicated into 0.01 M Tris buffer, pH 7.2 and centrifuged for 20 min. at 20,000 r.p.m. The particulate fraction was extracted with chloroform:methanol:water (2:2:1) and the amount of cholesterol determined spectrophotometrically by the method of Glick *et al.*¹⁴. Cholesterol (Sigma) was used as the standard and the extraction procedure was checked by thin layer chromatography using a chloroform:methanol:acetic acid (90:10:1) solvent. Protein determinations were done according to the Bradford method¹⁵. Phospholipid concentrations were measured from the extract of the particulate fraction by the colorimetric method of Raheja *et al.*¹⁶. A chromatographically pure reference standard of phosphatidyl choline (Sigma) was used.

The molar ratios of saturated to unsaturated fatty acid represents 18:0/18:1. Approximately 1.0×10^6 cells were used for this particular assay. After the particulate fraction was isolated (as previously described) 1 ml of 2.5 M KOH in methanol and 40 µg of heptadecanoic acid (an internal standard) were added and this mixture was heated for 30 min at 70 °C. After cooling, 1.5 ml of 1 M phosphoric acid was added and mixed well. Then 1 ml of hexane was added to the hydrolysate, mixed well, and centrifuged at 3,000 r.p.m. for 3 min. Two additional 0.5 ml hexane extractions were carried out and all the hexane solutions were combined. To the hexane solution, 200 µl of 0.5 M aqueous trimethyltrifluorotolylammonium hydroxide¹⁷ were added. Fatty acid methyl esters were formed on the gas chromatographic column (2 mm $\times$ 0.5 M, 3% Silar 10C on Gas Chrom Q 100/120) which was interfaced with a mass spectrometer (Finnigan 3300). The fatty acid methyl esters were quantified by their relative weight response to the internal standard using the total ion current of the selected ions.

Figure 1 shows the per cent survival of each cell line as a function of time at 43 °C. Note that all cell lines were grown in identical medium since it is known that exogenous cholesterol affects the cellular biosynthesis of the sterol¹⁸. Under these conditions, cellular sensitivity to a heat challenge is different for each cell line studied. HeLa cells appear to be the most thermal resistant cell line and CHO the most thermal sensitive of the lines tested. Also, most lines display a non-exponential survival response ('shoulder region') which precedes the exponential decrease in cell survival as time at 43 °C increases. The HeLa cells are an exception to this case, while the EMT6/AZ cells deviate from strictly exponential cell killing at longer treatment times. The plateau in the survival response for EMT6/AZ cells, after 200 min of hyperthermic exposure, represents the development of heat resistance and has been studied in detail for this line¹⁹.

Comparisons of the differences in thermal sensitivity between these lines can be made by determining the D_{01} , a common

Table 1 Cholesterol content in the particulate fraction of untreated cells growing at 37 °C and the sensitivity of these cells to 43 °C

Cell line	Source	Cholesterol (µg per mg protein)	D_{01} (43 °C) (min)
HeLa	Human cervix carcinoma	54.06 $\pm$ 1.50	54
Rat-1	Rat embryo fibroblast	33.90 $\pm$ 0.15	28
EMT6/AZ	Mouse mammary sarcoma	33.74 $\pm$ 0.98	24
RT-9	Rat astrocytoma	20.66 $\pm$ 2.86	12
CHO	Chinese hamster ovary	18.63 $\pm$ 0.41	10

parameter in the radiobiological literature, for each survival response. The D_0 value is simply the reciprocal slope of the exponential portion of the survival curve, and in this case, has units of time for a specific temperature. Thus, a survival curve with a higher D_0 than that of another indicates relative resistance to the particular treatment.

Table 1 lists the sources of the cell lines tested, the respective D_0 value for each cell line, which was calculated from Fig. 1, and the amount of cholesterol found in untreated cells. The quantity of cholesterol is expressed in terms of protein amounts found in the cell particulate fraction. There appears to be a remarkable correlation between D_0 and the cholesterol content. This correlation is still valid when the cholesterol content is related to cell number (data not shown). Since D_0 is inversely related to the survival sensitivity of treated cells, these data show that cholesterol content is inversely correlated with hyperthermic sensitivity. In addition, we investigated whether D_0 would also correlate with the cholesterol-to-phospholipid molar ratio or the degree of saturated to unsaturated fatty acid found in the particulate fractions of the cell lines under study. This is important since these various membrane parameters are also known to affect the physical state of the membrane and might just as easily correlate with the respective D_0 value. Table 2 gives the cholesterol-to-phospholipid molar ratio and the molar ratio of 18:0 to 18:1 observed in four of the nine cell lines tested. It is important to note that the ratio of saturated to unsaturated fatty acids does not follow any significant pattern with the amount of cholesterol present in each cell line. Our

Table 2 Relationship between heat sensitivity at 43°C, cholesterol:phospholipid molar ratio, and the ratio of saturated to unsaturated fatty acid (18:0/18:1)

Cell line	Heat sensitivity at 43°C (D_0) (min)	Cholesterol: phospholipid molar ratio	Saturated: unsaturated fatty acid molar ratio
Rat-1	28	0.264	1.4
EMT6/AZ	24	0.657	0.86
RT-9	12	0.470	1.0
CHO	10	0.502	1.4

interpretation is that the best correlate with the survival response to hyperthermia (D_0) is still the amount of cholesterol in the particulate fraction.

As described in Fig. 2, this correlation is expressed as a nearly linear relationship between the respective D_0 value for each cell line and the measurable cholesterol present in the cell particulate fraction. This linear relationship is impressive, since the thermal survival sensitivity does not correlate with any other obvious parameters of the lines tested, such as species, doubling time or colony-forming abilities, as mentioned earlier, of the untreated cells. Nor does survival sensitivity correlate directly with the tumorigenicity of the cell lines tested, since the Rat-1 and CHO cell lines are of normal tissue origins, while the HeLa, RT-9, and EMT6/AZ cell lines are of tumour origins. The RT-9 and EMT6/AZ cell lines are normally maintained by sequential *in vivo/in vitro* passage and form solid tumours when grown in F344 rats and BALB/c mice, respectively. It should be noted here that Green estimates that 95% of the cellular cholesterol is located in the plasma membrane²⁰. Therefore, it is reasonable to presume that the cholesterol content measured here represents plasma membrane cholesterol.

The demonstration of increased cholesterol content in cells that are relatively resistant to elevated temperatures (HeLa compared to CHO) is interesting because it suggests that the sterol provides a similar function to the saturation of fatty acids in prokaryotes. In addition, since hyperthermia is presently used as an anticancer agent, this finding offers the possibility of a

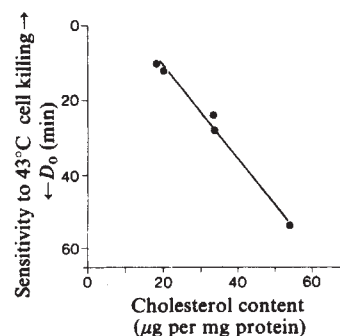


Fig. 2 Relationship between cholesterol content in untreated cells and 43°C survival sensitivity. The values for the respective D_0 and cholesterol content of each cell line were taken from Table 1. This emphasises in graphical form the apparent linear relationship between cellular sensitivity to 43°C and the cholesterol content in untreated cells.

predictive tool for determining individual tumour susceptibility to subsequent hyperthermia therapy.

In either case, it is important to test further the correlation of cholesterol, and other factors affecting membrane structure and permeability, and heat resistance. In this regard, it is of interest that the cholesterol:phospholipid molar ratios and the saturated:unsaturated fatty acid molar ratios do not correlate with various heat sensitivities of the cell lines tested. Studies to be published elsewhere do, however, show that the saturated:unsaturated fatty acid molar ratio does correlate with cell survival sensitivity at 43°C, within one cell line, when the normal growth temperature is either increased or decreased from 37°C. These studies emphasise that a number of factors affecting membrane structure and function may be involved in cellular responses to hyperthermia and that the normal growth temperature may be important in determining which of these factors are limiting. We are also extending the studies in this report by asking if cholesterol content is also elevated under conditions of induced thermal resistance¹². Preliminary experiments demonstrate that this is the case¹⁰, which in turn suggests that the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, should also be increased in thermal-resistant cells.

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The position of tropomyosin in muscle thin filaments

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The contraction of vertebrate skeletal muscle is controlled by the action of Ca^{2+} on muscle thin filaments. At low Ca^{2+} concentrations ($<10^{-6}$ M) the regulatory proteins of the thin filament, tropomyosin and troponin, relax muscle by preventing the interaction of myosin and actin. At higher Ca^{2+} levels this inhibition is removed, when Ca^{2+} binds to troponin. Tropomyosin, a long coiled-coil α -helical molecule, is located in each of the two long-pitch helical grooves of actin, but troponin, a globular molecule, is attached at intervals of 38 nm (refs 1, 2). By combining evidence from X-ray diffraction studies of muscle and from electron microscopy, several authors have proposed that tropomyosin moves to block or allow attachment of myosin heads to actin³⁻⁵. We investigate here an alternative way of combining the data, which if valid may have important consequences for our understanding of the regulation of muscle contraction⁶.

Figure 1 illustrates the problem. The shape and attachment site of myosin subfragment-1 (S-1) are based on a three-dimensional reconstruction of electron micrographs of the S-1-thin filament complex calculated by Moore *et al.*⁷. This reconstruction does not have sufficient resolution to reveal tropomyosin, but its position may be derived from analysis of X-ray diffraction patterns of muscle³⁻⁵, or image analysis of electron micrographs of reconstituted thin filaments^{8,9}. However, as pointed out by previous authors^{3,8}, these studies have not shown on which side of the actin groove tropomyosin is located relative to the S-1 attachment site. To remove this ambiguity, it is necessary to superimpose, with the correct relative orientation, reconstructions of decorated and undecorated thin filaments, the latter showing tropomyosin well resolved. If reconstructions are carried out on micrographs of I-segments, the assemblies of thin filaments still attached to the Z-line, the correct orientation is known, because the characteristic arrowhead appearance obtained by adding S-1 to thin filaments always points away from the Z-line¹⁰.

An I-segment from a crude muscle homogenate obtained at a low Ca^{2+} concentration is shown in Fig. 2a. Some homogenates at a high Ca^{2+} level were studied, but acto-myosin precipitation

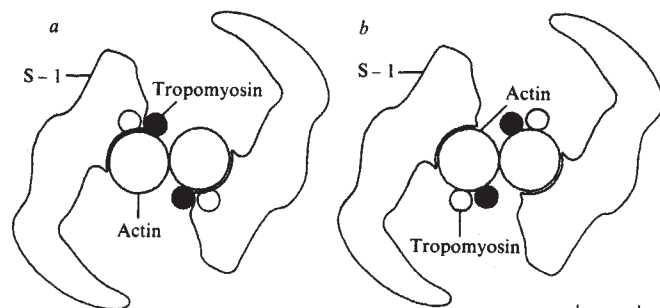


Fig. 1 The two ways of superimposing reconstructions from decorated (that is, complexed with S-1) and undecorated thin filaments. *a*, Based on Huxley³. *b*, As *a* except that the thin filament has the opposite orientation. The arrowheads are pointing out of the plane of the paper away from the Z-line. Tropomyosin in contracting muscle is represented by filled circles and in relaxed muscle by open circles. Scale bar, 5 nm.

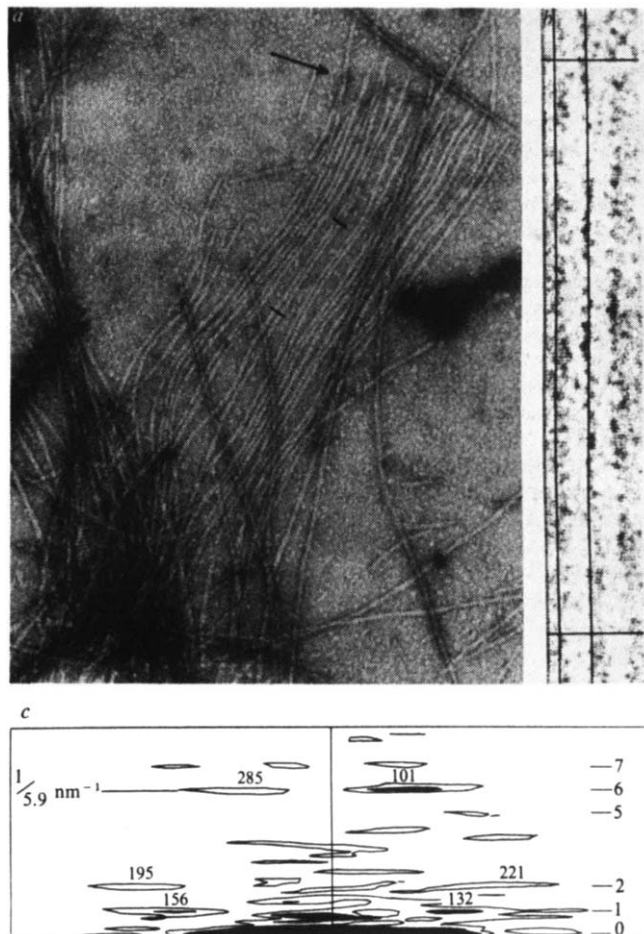


Fig. 2 *a*, Electron micrograph of an I-segment in a crude muscle homogenate at a low Ca^{2+} and high Mg^{2+} level. The arrow indicates the end of the I-segment 'brush'. The bars coincide with the narrow end of the rectangle shown in *b*. Preparations were obtained from homogenisation of rabbit psoas muscle in a relaxing medium (0.1 M KCl, 10 mM MgCl_2 , 6 mM phosphate buffer, 1 mM EGTA, 5 mM ATP and 0.5 mM dithiothreitol at pH 7.0 with $I \approx 0.14$). High Mg^{2+} concentrations were achieved by dilution or dialysis into the above relaxing medium, except KCl was replaced by MgCl_2 to give an overall $I \approx 0.15$. Some preparations were diluted or dialysed into a high Ca^{2+} concentration medium (0.1 M KCl, 6 mM MgCl_2 , 4 mM ATP, 2 mM CaCl_2 and 10 mM ImCl at pH 7.3 and $I \approx 0.13$). Specimens were mounted on carbon-coated copper grids and negatively stained with 1% uranyl acetate. Magnification $\times 88,000$. *b*, Display of densitometered region of *a*. The marked rectangle encloses the area Fourier transformed in *c*. *c*, Fourier transform of single filament shown in *b*. Amplitude peaks are contoured and numbers above layer-line peaks indicate phases. Layer-line numbers shown on right hand side are those arising from an actin filament with 13 subunits in 6 turns of the genetic helix⁷.

made these experiments difficult. At low Ca^{2+} concentrations, all preparations produced up to 80% inhibition of the acto-myosin ATPase rate, confirming the presence of regulatory proteins. Furthermore, troponin gave rise to strong meridional reflections at $1/38.5 \text{ nm}^{-1}$ in the Fourier transforms of many micrographs and also gave rise to corresponding periodic stripes visible in large I-segments. Side-by-side aggregation of filaments into paracrystals was induced by raising the Mg^{2+} concentration, but single-layered paracrystals of more than two or three filaments were rare because movement of the filaments was restricted by their Z-line attachment and tangling readily occurred. Filaments within paracrystals are usually better for image analysis studies because they are straighter, more evenly stained and less flattened¹¹.

Only filaments that seemed to be connected to a Z-line and terminated at the end of the I-segment 'brush' were considered for analysis. Optical diffraction patterns of electron micrographs of many hundreds of such filaments were examined, but only 20 patterns clearly showed at least three actin layer lines. The density distribution of these micrographs was converted into digital form and their Fourier transforms calculated¹¹ (Fig. 2*b, c*). The quality of the phase and amplitude information justified three-dimensional reconstruction in eight cases, where for example, the phase residual was always less than 37° and had an average value of 28°. The phase residual (see Table 1) is a measure of the amount by which the helical symmetry deviates from that expected for actin⁷, and was calculated for all non-equatorial layer-line data of medium or strong intensity. A high phase residual was associated with asymmetries between the upper and lower sides of the filaments and the relatively poor signal:noise ratio, arising from additional proteins in the homogenate and filament curvature restricting the length of filaments processed. Straight filaments not in paracrystals sometimes gave low phase residuals, but in most cases it was better to mask off a filament within a paracrystal or to deconvolute the Fourier transform of the paracrystal to give the transform of an average filament. Six reconstructions clearly resolved the relative positions of actin and tropomyosin, in one case the Ca²⁺ level was high. By orientating each reconstruction with respect to the Z-line, tropomyosin was consistently found to occupy positions shown in Fig. 1*b*, that is, on the opposite side of the actin groove to that of the postulated S-1 binding site.

Two reconstructions can be calculated from any set of Fourier transform data, using layer-line information arising from either the upper or lower sides of the filament¹². In Table 1 the discrepancies between the peak amplitudes measured on the left and right hand sides of the meridian and the sometimes large

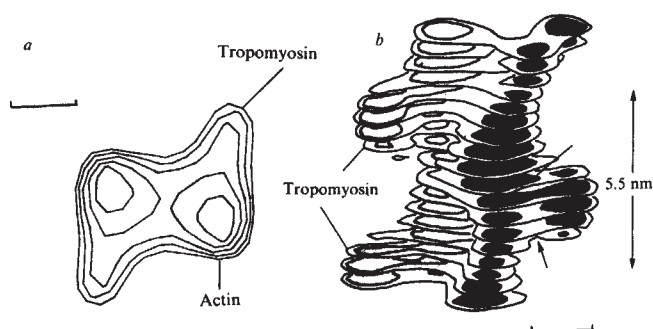


Fig. 3 *a*, Contour map of the helical projection derived from averaged layer-line data. The helical projection is formed by projecting the structure down the long-pitch (72 nm) actin helices looking towards the Z-line. *b*, Three-dimensional reconstruction from averaged layer-line data, with the Z-line at the bottom. The different shadings indicate the long-pitch actin and tropomyosin strands on opposite sides of the helical axis. Actin layer lines⁷ used in these reconstructions included $l = 0, 1, 2, 3, 5, 6, 7$ and 8 . The equator was used at full weight and meridional reflections on the first layer line were weighted down⁸. Data were derived from three transforms originating from filaments at low Ca²⁺ concentrations. Scale bars, 2 nm.

Table 1 Peak amplitude and phase data

Filament reference no.	Actin layer-line no. ⁷	Peak amplitudes (arbitrary scale, not normalised)		$\Delta\theta \mp \pi n$	Phase residual
		LHS	RHS		
1	1	115	217	36°	28°
	2	108	52	32°	
	6	172	165	9°	
2	1	164	245	4°	20°
	2	76	151	28°	
	6	172	137	1°	
3	1	143	162	4°	25°
	2	85	125	88°	
	6	117	141	8°	
4	1	129	201	14°	37°
	2	83	94	11°	
	6	141	147	27°	
5	1	187	167	18°	24°
	2	119	127	29°	
	6	203	118	11°	
6	1	98	96	56°	31°
	2	75	79	1°	
	6	73	124	46°	

The data for filament 2 were obtained from the deconvoluted Fourier transform of a paracrystal. In other cases, the data shown are from the transform of masked-off filaments. For filaments 4 and 5, the deconvolution analysis was also carried out and gave results not significantly different from those shown. $\Delta\theta$ is the phase difference between Fourier coefficients along a layer line on opposite sides of the meridian and at a radial spacing equal to the average radial spacing of the peak amplitude on each side of the meridian. n is the principal Bessel order expected on the layer line in question. The phase residual is normalised, amplitude weighted ($\Delta\theta \pm \pi n$) using all Fourier coefficients whose amplitudes are significantly above the background noise level, as described by Wakabayashi *et al.*⁸ (where it is termed a 'self residual' Q). The full amplitude contour map for filament 5 is shown in Fig. 2*c*.

value of ($\Delta\theta \mp \pi n$) indicate a difference in the stain distribution on the two sides of the filament. The data corresponding to each side were therefore used independently to calculate three-dimensional reconstructions, and in each case the assignment of the actin and tropomyosin positions was the same. Additionally, model calculations indicated that phase changes on the first and second layer lines of two to three times the average phase residual would be required to move the tropomyosin from one side of the actin groove to the other.

Five sets of data from three filaments (2, 3 and 5 of Table 1) were averaged by the methods of Amos and Klug¹³, and Fig. 3*a* shows the resulting helical projection. The averaging produced an improvement in the signal:noise ratio of the data, particularly on weaker layer lines. Great care was taken to preserve the known orientation of each filament and the effect of each mathematical operation was monitored on a test object, which was an S-1-decorated thin filament having an obvious polarity. The helical projection shows on each side a well resolved actin peak with, extending to higher radius, a smaller region of lower density attributable to tropomyosin. This assignment is confirmed by the three-dimensional analysis (Fig. 3*b*), where the size and shape of the actin subunit are consistent with that observed in other reconstructions⁸. In common with earlier reconstructions¹⁴, tropomyosin seems to be discontinuous, which may be due to inadequate resolution and the periodic merging of tropomyosin with part of the actin subunit. Where tropomyosin diverges from one end of the actin subunit a V-shaped cleft is formed (arrow, Fig. 3*b*). Furthermore, each actin subunit makes a connection to each of the two adjacent subunits in the other long-pitch strand and these are asymmetrically disposed with respect to the subunit's centre of mass. These features are present in the reconstructions of Wakabayashi *et al.*⁸ and assist in assigning filament polarity.

These reconstructions remove an important ambiguity present in all previous structural work as to which side of the actin groove tropomyosin occupies. It is not known whether the azimuthal movement of tropomyosin regulates contraction by sterically blocking incoming myosin heads, or by changing the conformation of actin subunits so that they cannot bind myosin. The steric blocking model was formulated on the assumption that tropomyosin occupies positions shown in Fig. 1*a*, which we have found to be erroneous. In Fig. 1*b* there is no obvious way in which tropomyosin can sterically block myosin, unless the shape

of S-1 or its position of attachment to actin is revised. We understand that K. Taylor and L. A. Amos (personal communication) are now re-examining the structure of decorated thin filaments and their results will be of particular interest.

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Electron transfer reagent binding sites on plastocyanin

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Electron transfer is an essential process in all biological systems. The kinetics of electron transfer reactions between small inorganic complexes and a variety of metalloproteins have been extensively studied^{1–5}. Recent studies on the electron transfer reactions of plastocyanin, an essential component of the photosynthetic electron transport chain, have shown^{6–9} that inorganic redox reagents such as the hexacyanoferrate(III) ion ($\text{Fe}(\text{CN})_6^{3-}$) and the tris(1,10-phenanthroline)cobalt(III) ion ($\text{Co}(\text{1,10-phen})_3^{3+}$) form discrete complexes with the protein before the electron transfer step. Here, we have used high resolution nuclear magnetic resonance spectroscopy (NMR) to study the interaction of plastocyanins from French bean (*Phaseolus vulgaris*) and cucumber (*Cucumis sativus*) with the chromium(III) analogues of these redox reagents, $\text{K}_3\text{Cr}(\text{CN})_6$ and $\text{Cr}(\text{1,10-phen})_3(\text{ClO}_4)_3$. The binding of these complexes to plastocyanin is highly specific and we identify two binding sites, separated by about 15 Å, which are apparently determined primarily by electrostatic interactions. Two distinct electron transfer pathways to and from the copper atom are indicated.

Plastocyanin is a type 1 or 'blue' copper protein of molecular weight 10,500. It contains one copper atom, in a distorted tetrahedral environment, which is coordinated by the thiol group of Cys 84, the thioether group of Met 92 and the imidazole groups of His 37 and His 87 (ref. 10). Its low molecular weight makes it ideally suited to study by high resolution NMR spectroscopy and to date the resonances of many amino acids near the copper atom have been identified^{11–14}. These resonances, which are readily detected in the spectrum of the copper(I) protein, are broadened beyond detection when the protein is in the copper(II) state. The aromatic region of the proton NMR spectrum of reduced French bean plastocyanin is shown in Fig. 1a. The resonances of both histidine residues, all three tyrosine and two phenylalanine residues have been identified previously¹³. With the help of the X-ray crystal structure¹⁰, assignments to specific amino acids can now be made. Full details will be published separately but we note here that coupled doublets at 6.51 and 6.64 p.p.m., which arise from the tyrosine residue closest to the copper atom¹³, can, from the

X-ray structure¹⁰, be assigned to Tyr 83. Specific assignments of the histidine resonances have also been made. On the basis of their pronounced pH dependence (ref. 11 and our own unpublished observations), resonances at 7.66 and 6.97 p.p.m. are assigned to the C-2 and C-4 protons¹³, respectively, of His 87 which is exposed to the solvent at one end of the molecule¹⁰. The other, more deeply buried histidine (His 37) must thus give rise to the resonances¹³ at 7.57 and 7.16 p.p.m. which show only slight dependence on pH. The spectrum of the French bean protein has many features in common with those of other plastocyanins and the protein structure, particularly in the vicinity of the copper atom, is expected to be conserved in plastocyanins from all higher plant species¹³.

The effect of addition of $\text{Cr}(\text{CN})_6^{3-}$ to a solution of reduced French bean plastocyanin is shown in Fig. 1b. Significant changes in the NMR spectrum occur and are best seen in the difference spectrum shown in Fig. 1c. At low concentrations of $\text{Cr}(\text{CN})_6^{3-}$, the resonances of His 87 broaden. As the concentration of $\text{Cr}(\text{CN})_6^{3-}$ is increased, the resonances of His 37 also begin to broaden. In the aliphatic region of the spectrum (not shown), the resonance of Met 92 (a copper ligand) at 0.57 p.p.m. is broadened slightly.

Perturbations are also observed in the NMR spectrum of reduced plastocyanin when the protein is titrated with $\text{Cr}(\text{1,10-phen})_3^{3+}$ (Fig. 2). However, these perturbations differ from those observed with $\text{Cr}(\text{CN})_6^{3-}$. The resonances of Tyr 83 are broadened dramatically, the effect being most noticeable at a $\text{Cr}(\text{1,10-phen})_3^{3+}$ concentration of 0.4 mM (3.5 mM plastocyanin). In the aliphatic region of the spectrum, the methyl group proton resonances of two or three (as yet unidentified) amino acid residues are broadened. These resonances are not broadened by $\text{Cr}(\text{CN})_6^{3-}$.

The different perturbations of the NMR spectrum induced by $\text{Cr}(\text{CN})_6^{3-}$ and $\text{Cr}(\text{1,10-phen})_3^{3+}$ indicate that these reagents bind to plastocyanin at two different sites. The broadening of resonances results from an enhancement of nuclear relaxation brought about by dipolar coupling with the relatively slowly relaxing electron spin of the Cr(III) ion. The broadening is dependent on the inverse 6th power of the distance of the amino

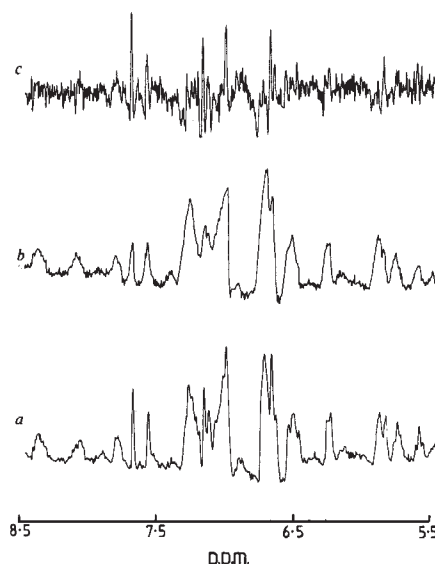


Fig. 1 Aromatic region of the resolution-enhanced NMR spectrum of French bean plastocyanin (3.5 mM, pH 6.3, 50 mM phosphate buffer in D_2O) in the presence of no $\text{Cr}(\text{CN})_6^{3-}$ (a) and 0.4 mM $\text{Cr}(\text{CN})_6^{3-}$ (b). c, Difference spectrum (a – b). In a and b the resolution has been enhanced by means of the convolution difference technique¹⁶. The residual HOD peak was suppressed by selective gated irradiation. Dioxan was used as an internal standard but all peaks are referred to TSS (trimethylsilylpropanesulphonic acid). The pH values quoted are uncorrected meter readings. Plastocyanins were prepared by a modification of the method of Ramshaw *et al.*¹⁷.

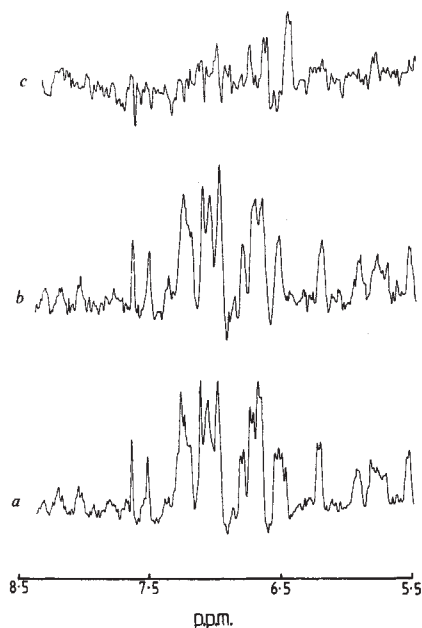


Fig. 2 Aromatic region of the resolution-enhanced NMR spectrum of cucumber plastocyanin (3.5 mM, pH 7.0, 50 mM phosphate buffer in D_2O) in the presence of no $Cr(1,10-phen)_3^{3+}$ (a) and 0.4 mM $Cr(1,10-phen)_3^{3+}$ (b). c, Difference of direct spectra obtained before and after addition of 0.4 mM $Cr(1,10-phen)_3^{3+}$.

acid residue(s) from the paramagnetic centre. Consequently, any resonances broadened by the addition of the $Cr(III)$ complexes must arise from amino acid residues close to the sites of binding to the protein. One site, to which $Cr(CN)_6^{3-}$ binds, must lie close to the copper atom itself, as the resonances of three of the copper ligands are broadened. The resonances of His 87 are observed to broaden first. The copper atom lies at one end of the protein molecule in a depression lined by conserved hydrophobic residues and is protected from solvent by the imidazole group of this histidine residue¹⁰. It is conceivable that positive charge from the copper atom is delocalised over this imidazole ring which would be sufficient to bring about an electrostatic interaction with $Cr(CN)_6^{3-}$.

The second site must lie in the vicinity of Tyr 83 because the resonances of this residue are broadened by the binding of $Cr(1,10-phen)_3^{3+}$. The side chain of Tyr 83 lies close to the surface of the protein about 10 Å from the copper atom and is adjacent to a cluster of carboxylate groups¹⁰ belonging to conserved acidic residues. This 'pocket' of negative charge is unique in plastocyanin and clearly provides a favourable binding site for positively charged reagents. Our NMR studies show that $Cr(NH_3)_6^{3+}$ also binds at this site (D.J.C., M.T.H. and P.E.W., unpublished observations).

Our results suggest that the charge on the inorganic reagent has a dominant role in determining the site of interaction on the protein. Sykes and coworkers⁹ have shown that oxidation reactions involving $Co(1,10-phen)_3^{3+}$ are blocked by $Cr(1,10-phen)_3^{3+}$, indicating conclusively that these reagents compete for a single binding site on the protein from which electron transfer occurs. Furthermore, we expect $Cr(CN)_6^{3-}$ to bind at the same site as $Fe(CN)_6^{3-}$ in view of their similar size and identical charge and ligands. The existence of two binding sites for redox reagents on plastocyanin is not unexpected in view of the electron transport function of the protein¹⁵.

The rate constants for the reactions of parsley plastocyanin with $Fe(CN)_6^{3-}$ and $Co(1,10-phen)_3^{3+}$ are both pH dependent but have different pK_a values (5.7 and 6.1, respectively)^{7,9}. In addition, the reciprocal of the first order rate constant, k_{obs} , is inversely proportional to the oxidant concentration⁷. These observations led Sykes and coworkers to conclude that productive complexes are formed before electron transfer and that at least two binding sites are involved which they supposed to be in the same locality of the protein⁹. In our present NMR studies we

have identified these two binding sites on plastocyanin. One is close to the copper atom and the other is at least 10–15 Å away. This observation confirms earlier suggestions based on the X-ray crystal structure¹⁰ that two electron transfer pathways to and from the copper atom are used in the redox reactions of plastocyanin and that possibly two mechanisms of electron transfer are involved.

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The β -globin gene is on the short arm of human chromosome 11

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Investigations on the regulation of human globin gene expression are assisted by a knowledge of the chromosomal location of the globin genes. Previous studies¹ have mapped the α -globin locus to human chromosome (HC) 16, and have shown that the human globin gene complex γ - δ - β co-segregates with lactate dehydrogenase A (LDH-A) and the presence of HC 11 in somatic cell hybrids^{2,3}. Radioactively labelled globin complementary DNA (cDNA) probes were used in molecular hybridisation experiments to determine the chromosomal locations of the α - and β -globin genes. When human $\times$ rodent somatic cell hybrids are used which contain well-defined parts of human chromosomes, direct mapping of genes of chromosomal regions or single bands is possible^{4,5}. We have regionally localised the human β -globin gene using two sets of hybrid cell lines: (1) Chinese hamster $\times$ human hybrid cells containing the HC 11 long arm or both the short and long arms and (2) mouse $\times$ human hybrids containing only the HC 11 short arm. The techniques of liquid molecular hybridisation³ and Southern blotting⁶ with ³²P-labelled human β -globin cDNA (from plasmid JW102)⁷ have been used to localise the β -globin gene sequence to region 11p11 $\rightarrow$ 11p15. Similar results were reported recently by Jeffreys *et al.*⁸.

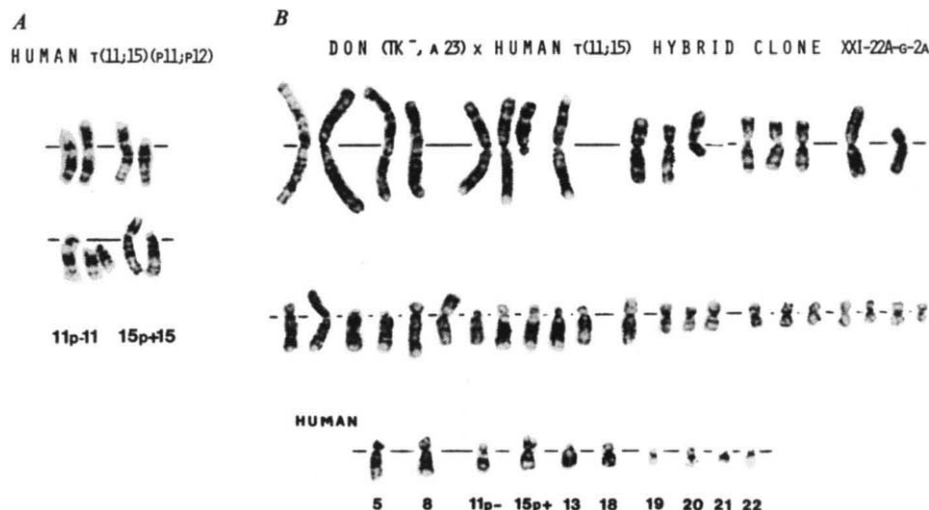


Fig. 1 A, Chromosome pairs 11 and 15 showing balanced reciprocal translocation $t(11;15)(p11;p12)$. The rearranged chromosomes $der(11)$, or $11p-$, and $der(15)$, or $15p+$, are placed to the left and the normal homologues 11 and 15 to the right of each pair. Trypsin-Giemsa banding was carried out according to Francke and Oliver¹². B, Karyotype of Chinese hamster (Don, TK⁻) x human $t(11;15)$ hybrid clone with trypsin-Giemsa banding. The human chromosomes are arranged in the bottom line. Both translocation chromosomes are present but the normal chromosome 11 has been lost.

Chinese hamster x human cell hybrids containing the long arm of HC 11 were obtained when skin fibroblasts from a balanced carrier of a 46, XY, $t(11;15)(p11;p12)$ translocation (Fig. 1A) were fused to Chinese hamster Don cells (a23; TK⁻) using polyethylene glycol as a fusogen⁹. Cell hybrids were selected in hypoxanthine/aminopterin/thymidine (HAT) medium. The translocation chromosomes $der(11)$ or $11p-$ (region 11p11 → qter) and $der(15)$ or $15p+$ (region 11pter → p11) and the normal HC 11 segregated randomly. After screening many primary hybrid clones for their content of chromosomes containing HC 11 material, and after two rounds of subcloning, we have isolated a number of independent primary and secondary hybrid cell lines which have retained the $der(11)$ chromosome at high frequency and have lost the normal HC 11 and the $der(15)$ translocation product. These clones do not express human LDH-A, while others containing both translocation chromosomes after loss of the normal HC 11 (Fig. 1B), are positive for LDH-A expression. These results are consistent with the previous localisation of LDH-A to band 11p12 (ref. 10).

Mouse x human somatic cell hybrids containing the short arm of HC 11 were obtained when BALB 3T3 cells deficient in

thymidine kinase (TK) were fused to human fibroblasts with a $t(11;17)(p15;q21)$ balanced translocation. In subclones a secondary rearrangement was identified and characterised as $11cen \rightarrow 11p15::17q21 \rightarrow 17qtr$ (mar-1). This telocentric marker chromosome carries the human gene for TK (in region 17q21 → 17qtr) and the gene for LDH-A (in region 11cen → 11p15)⁴. This chromosome is selectively retained in cell hybrids grown in HAT medium because it carries the human TK gene, complementing the enzyme defect in the murine parental cell line. In fact, mar-1 was the only human chromosome retained in hybrid clones VII-2,3 and 4 HAT, as demonstrated by Hoechst 33258 and Giemsa 11 staining. (A cell line derived from one of these hybrid clones has been designated LBP-2 in the laboratory of Dr Walter Bodmer and has been used by Jeffreys *et al.* in similar experiments to those described here⁸.) After counter selection in 5-bromodeoxyuridine (BUdR)-containing medium, derivative clones lacking mar-1 have been obtained (VII-2 and 4, BUdR)¹¹. These back-selected clones no longer express LDH-A.

Hybrid clones from both sets of experiments were characterised cytologically by trypsin-Giemsa banding and karyotyping (Fig. 1A, B), and biochemically by LDH electrophoresis

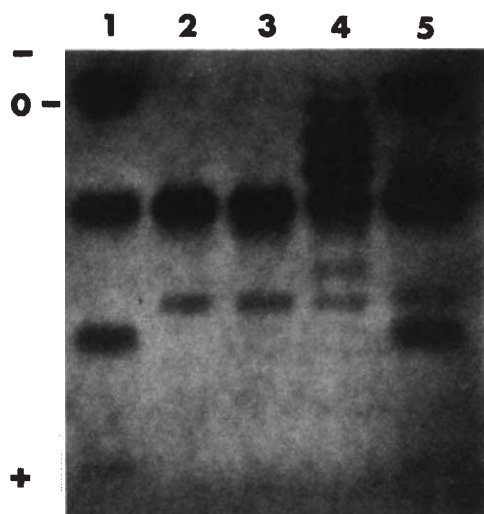
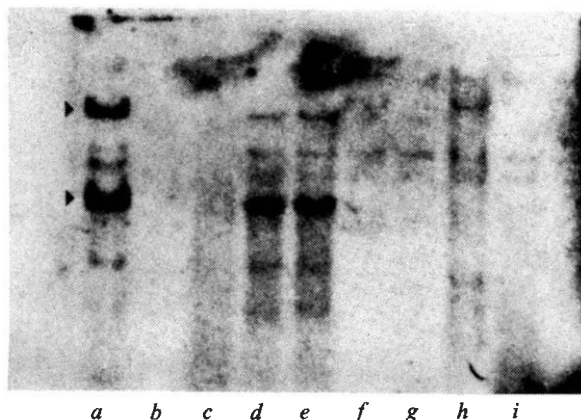


Fig. 2 Lactate dehydrogenase electrophoresis on Cellogel¹³. Cell extracts: 1, Human fibroblast; 2, mouse 3T3/TK- parental cells; 3, hybrid clone VII-2 BUdR; 4, hybrid clone VII-2 HAT; 5, mixture of extracts (1) and (2). The hybrid in channel 3 is negative and the one in channel 4 is positive for human LDH-A.

Table 1 Properties of clones used in this study

	Human chromosomes*			Human LDH-A	β Globin by Southern blot	β Globin by liquid molecular hybridisation
<i>Chinese hamster x human</i>	<i>der(11)</i>	<i>der(15)</i>	<i>11</i>			
XXI-61A	0.5	0	0	-	-	-
XXI-22A-c	0.9	0	0	-	-	-
XXI-22A-c-2b	0.8	0	0	-	-	-
XXI-22A-c-2c	1.0	0	0	-	-	-
XXI-22A-g	0.7	0.7	0	+	+	+
XXI-22A-g-1a	1.0	1.0	0	+	+	+
XXI-22A-g-2a	1.0	0.8	0	+	+	+
XXI-22A-g-2b	0.8	0	0	-	-	-
XXI-23A-2d	1.4	0	0	-	-	-
<i>Control DNA</i>						
Human spleen	0	0	1.0	+	+	+
Don cell	0	0	0	-	-	-
	mar-1 (11 cen → p15)					
<i>Mouse x human</i>						
VII-2 HAT	0.9	0	+	+	+	+
VII-3 HAT	1.0	0	+	+	+	+
VII-2 BUdR	0	0	-	-	-	-
VII-4 BUdR	0	0	-	-	-	-
<i>Control DNA</i>						
Human spleen	0	1.0	+	+	+	+
Mouse liver	0	0	-	-	-	-

*Between 15 and 30 metaphase cells per clone were analysed. Results are presented as average frequency of respective chromosome per cell.



NaCl, 1 mM EDTA). The largest fragment, which contains the β -globin insert was collected by ethanol precipitation. This fragment was then incubated in the presence of ^{32}P -dCTP, DNase and *E. coli* PolI for nick translation^{3,14}. The DNA was extracted with phenol, collected in the void volume peak on a Sephadex G-50 column, and precipitated with ethanol. The resulting double-stranded probe had a specific activity of approximately $2\text{--}4 \times 10^6$ c.p.m. μg and was used for the Southern blotting procedure⁶. The lyophilised cDNA probe was resuspended in the prehybridisation solution described above, boiled to denature it, and added to the nitrocellulose filters in a Scotchpak bag. Hybridisation was carried out at 65°C for 72 h. After hybridisation, the blots were washed in several changes of prewarmed SSC, and autoradiographed with intensifying screens at -80°C for 48 h. Upper and lower arrows indicate *Eco*RI human β -globin fragments of 6.2 and 4.0 kilobases, respectively. These correspond to the 5.2 and 3.2 kilobase fragments reported by Lawn *et al.*¹⁵. The large amount of DNA loaded on the gel causes these fragments to migrate more slowly. a, Human spleen DNA; b, XXI 61A; c, XXI 23A-2d; d, VII-2 HAT; e, VII-3 HAT; f, VII-2 BrdU; g, VII-4 BrdU; h, Don cell DNA; and i, mouse liver DNA.

(Fig. 2). DNA was extracted from each cell for liquid molecular hybridisation or Southern blotting to detect human β -globin gene sequences.

All hybrid cell clones were expanded to 5×10^8 cells, and an aliquot of each was examined for LDH-A. The LDH-A analysis was used as a marker to monitor the presence of the short arm of HC 11. The remainder of the cell pellet was extracted for DNA as described in the legend to Fig. 3.

For Southern blotting, a 100- μg sample of each hybrid DNA was digested with *Eco*RI, electrophoresed in 1% agarose, blotted on to nitrocellulose filters and annealed in stringent conditions to ^{32}P -labelled pure human β -globin cDNA (obtained from the recombinant cDNA plasmid JW102). Thirteen hybrid clones were analysed (Fig. 3; Table 1). Two bands which hybridised strongly to human β -globin cDNA were present in the human spleen control DNA (Fig. 3, lane a). Several lighter bands were present in all DNA samples including the Chinese hamster (Fig. 3, lane h) and mouse liver (Fig. 3, lane i) control DNA, and probably represent cross-reactivity of the human β -globin cDNA with other sequences. The five clones positive for LDH-A were also positive for the human β -globin gene (Fig. 3, lanes d, e; Table 1); the eight clones negative for LDH-A were also negative for the human β -globin gene (Fig. 3, lanes b, c, f, g; Table 1).

Liquid molecular hybridisation was carried out, as described previously³, on six hybrid clones (Table 1, Fig. 4). Two were positive for LDH-A and the human β -globin gene; four were negative for both.

Thus, in both the Southern blot and the liquid molecular hybridisation assays, those hybrids containing the short arm of HC 11 and expressing human LDH-A were positive for the presence of the human β -globin gene, while those clones containing only the long arm of HC 11 were negative for the β -globin gene. Since p15 is the breakage point of mar-1, and 11p11 the breakpoint in the t(11;15) translocation, the β -globin gene complex can be limited to region 11p11 $\rightarrow$ 11p15 of the human chromosome.

These mapping studies may be of significance in the study of regulatory phenomena involving the γ - δ - β globin loci. Since in mar-1 the short arm of HC 11 (containing the β -globin gene region) is attached to HC 17 (containing thymidine kinase) this

Fig. 3 Southern DNA blotting of hybrid clones and controls. Hybrid cells were collected for karyotyping, LDH-A analysis, and DNA extraction all from the same (or from a very close) passage. The cells were pelleted, and resuspended in 50 mM EDTA, 0.5% SDS, and 100 $\mu\text{g ml}^{-1}$ proteinase K (Beckman). DNA extraction proceeded by addition of an equal volume of 1:1 chloroform:phenol, separation of the phases, addition of an equal volume of chloroform:isoamyl alcohol (24:1) to the aqueous phase, separation of the phases, and two ethanol precipitations. The nucleic acid was pelleted at 10,000 r.p.m. for 45 min, and resuspended in SSC (0.15M NaCl + 0.015 M Na citrate, pH 7.0). RNase (previously boiled for 10 min to inactivate residual DNase) was added to a concentration of 100 $\mu\text{g ml}^{-1}$ and incubated at 37°C for 1 h. Extraction with phenol:chloroform was performed, followed by a second ethanol precipitation. The DNA pellet was resuspended in deionised water and dialysed for 2 d in Tris-EDTA (100 mM Tris-HCl, pH 7.2, 0.5 mM EDTA). 100- μg samples of each hybrid DNA were digested for 2 h at 37°C with *Eco*RI (Boehringer Mannheim) 2 units per μg , and electrophoresed in 1% agarose. The DNA was blotted on to nitrocellulose filters in $6\times\text{SSC}$. The blots were prehybridised in 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 50 $\mu\text{g ml}^{-1}$ denatured salmon sperm DNA, 10 $\mu\text{g ml}^{-1}$ poly(A) and 0.1% SDS to prevent nonspecific hybridisation of the β -globin probe. The cDNA probe was prepared from plasmid JW102⁷ which was digested with *Hha*I and separated by size on an alkaline sucrose gradient (5–20% sucrose in 10 mM Tris-HCl, pH 7.5, 100 mM

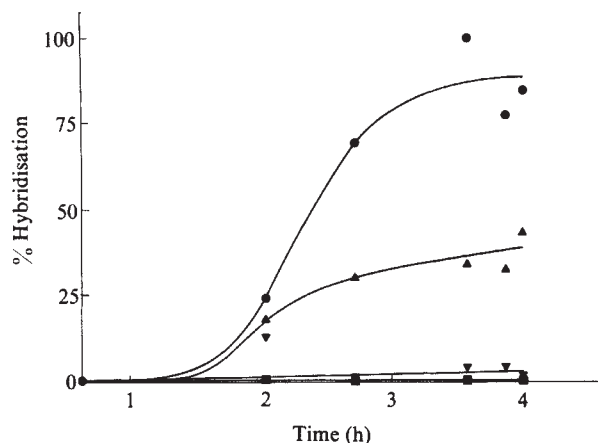


Fig. 4 Liquid molecular hybridisation of hybrid cell DNA to ^{32}P -labelled human β -globin cDNA. Hybrid cells were pelleted and extracted for DNA as described previously³. The double-stranded ^{32}P -labelled β -globin cDNA probe, prepared as described in the legend to Fig. 3, was further purified by hybridisation to adult human reticulocyte mRNA in 50% formamide and $3\times\text{SSC}$ at 66.5°C . This temperature prohibits DNA duplex formation but allows DNA-mRNA hybridisation, which then protects the antimesage strand from subsequent digestion with S_1 nuclease. After addition of *E. coli* carrier DNA and alkaline buffer, the duplex was separated on a 5–30% alkaline sucrose gradient. The hot peak of 500–800 nucleotide material was pooled, neutralised, and precipitated in ethanol. The precipitate was dissolved in deionised water and desalted over a Sephadex G-25 column. The purity of the ^{32}P -labelled, single-stranded β -globin specific cDNA was tested by annealing to probe at increasing concentrations of adult human reticulocyte mRNA. For detection of the human β -globin gene in hybrid DNA, the sonicated DNA of each cell line was lyophilised in 3-mg aliquots and mixed with a cocktail of 50% formamide, $3\times\text{SSC}$, and ^{32}P -labelled probe. The reaction was divided into 20- μl aliquots and sealed in glass capillary tubes coated with *E. coli* DNA to minimise sticking. All reactions were boiled for 15 min to denature the DNA and allowed to reanneal at 52.5°C . Time points were taken from 10 min to 100 h to generate the C_0t curve. All time point samples were divided, and one-half of each digested with S_1 nuclease to determine the per cent hybridisation of each sample. (●) Human spleen DNA; (▲) XXI 22A-g; (▼) XXI 22A-g-2b; (■) Don cell DNA.

translocation product may be selectively retained in cell hybrids maintained in HAT medium. Fusion of VII-2 HAT with TK⁻ 2S mouse erythroleukaemia cells³ should result in cell hybrids in which the human β -globin gene is maintained in an established mouse erythroid tissue culture cell line. Studies are now under-way to obtain this proposed hybrid line.

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Satellite DNA is transcribed on lampbrush chromosomes

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During the lampbrush stage of oogenesis there is widespread transcription, and it has been estimated that the total amount of DNA transcribed may be an order of magnitude greater than that required to produce the necessary functional RNA for the oocyte¹. We therefore considered it likely that some of the transcribed sequences have little, if any, translational significance, and may include both middle repetitive² and highly repeated, or satellite, sequences. Satellite DNA is generally defined as rapidly reannealing DNA which has a short basic sequence that is repeated millions of times in the genome, usually in tandem arrays^{3,4}. The short repeated length, coupled with the organisation of satellite sequences in high order molecular weight tandem arrays in heterochromatic regions, have been put forward as reasons for supposing that this type of DNA is not normally transcribed⁵. We report here that we have looked for and found evidence of transcription of satellite DNA on lampbrush loops in oocytes of the crested newt, *Triturus cristatus cristatus*.

The technique that we have used to detect transcription is that of DNA/RNA-transcript hybridisation⁶, where a radioactively labelled DNA probe is hybridised to nascent RNA transcripts on the lampbrush loop. Preliminary studies using this approach⁷ indicated that highly repeated DNA sequences are transcribed around some lampbrush loops during oogenesis in *T.c. cristatus*. However, the probe used in these experiments undoubtedly

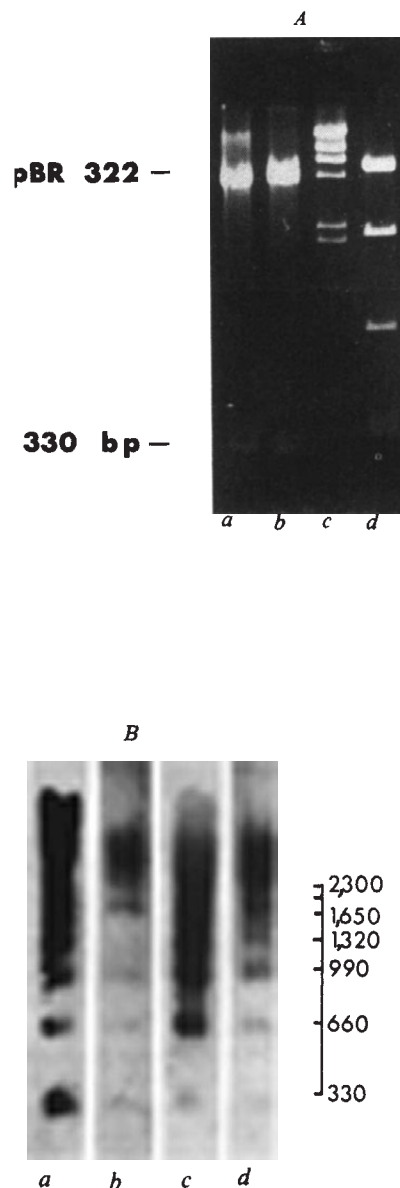


Fig. 1 A, Analysis of recombinant plasmids pTcS1 and pTcS2 by restriction endonuclease cleavage and electrophoresis on a 1% agarose gel. pTcS1 and pTcS2 were digested with *Bam*HI (tracks a, b). Linear pBR322 and a 330-base pair fragment are resolved as indicated. Tracks c and d show *Hind*III digests of λ and PM2, respectively. pTcS1 and pTcS2 had been produced by ligating pBR322 cleaved with *Bam*HI and the partially purified *Bam*HI 330-base pair satellite sequence. Clones containing plasmids that failed to confer tetracycline resistance were grown in M9 medium containing 100 $\mu\text{g ml}^{-1}$ ampicillin at 37°C. The plasmids were amplified using chloramphenicol (250 $\mu\text{g ml}^{-1}$)¹⁸ and covalently closed circular plasmid DNA was isolated as supercoils by lysozyme–Brij 58–Na deoxycholate lysis of the cells¹⁹ followed by centrifugation in caesium chloride–ethidium bromide. Plasmids containing *T.c. cristatus* DNA were propagated under P2 containment as defined by the NIH. B, Autoradiographs of nitrocellulose filters which have been hybridised to pTcS1 and pTcS2 nick translated with [α -³²P]dCTP (Radiochemical Centre; Code no. PB 205; specific activity 2–3,000 Ci mmol⁻¹). Tracks a and c represent transfers of total *T.c. cristatus* DNA digested with *Bam*HI, tracks b and d represent total *T.c. cristatus* DNA digested with *Kpn*I, which also cleaves once within this 330-base pair repeat. Tracks a and b have been hybridised with pTcS1, tracks c and d with pTcS2. In each case, a ladder of sequences representing multimers of the 330-base pair repeat hybridise to the cloned fragments, showing that the cloned fragment represents a tandemly repeated satellite sequence. Autoradiography was carried out for 2–5 days at –20°C using an Ilford fast tungstate intensifying screen.

contained some middle repetitive and unique DNA sequences, which could have been responsible for the results obtained. To determine whether satellite DNA is transcribed during oogenesis, we have isolated a 330-base pair satellite sequence from total *T.c. carnifex* DNA by digestion with the restriction endonuclease *Bam*HI. This restriction fragment was inserted into the *Bam*HI site in the tetracycline resistance gene in pBR322 (ref. 8). Plasmid DNA was purified from cultures of *Escherichia coli* K12 strain HB101 (ref. 9) containing the recombinant plasmid, and digested with *Bam*HI. After agarose gel electrophoresis, bands corresponding to linear pBR322 and to a sequence 330 base pairs long (Fig. 1A) were visible. To confirm that the 330-base pair sequence was a monomer of a tandemly repeated satellite, two of the recombinant clones, pTcS1 and pTcS2, were labelled *in vitro* with [α^{32} P]dCTP. These probes were then hybridised to total *T.c. carnifex* DNA

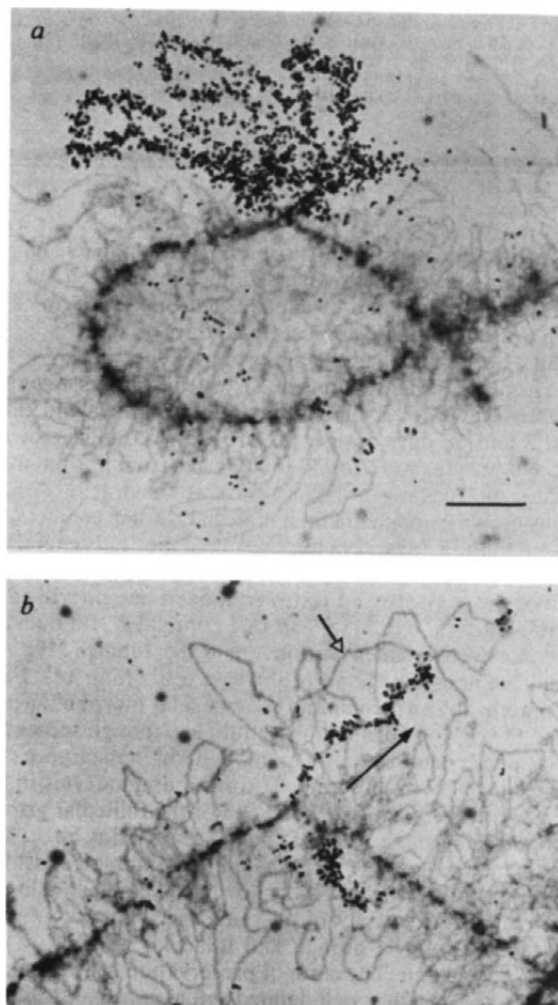


Fig. 2 Light micrographs of DNA/RNA-transcript hybrids of 3 H-labelled pTcS1 DNA onto lampbrush loops of *T.c. carnifex*. Lampbrush chromosomes were isolated as described previously¹², but were fixed in 70% ethanol only. Hybridisation was carried out in 50% formamide, $4 \times$ SSC at 37°C for 12 h (ref. 2). The specific activity of the probe was 2×10^6 c.p.m. per μg and $1-2 \times 10^5$ c.p.m. were used per preparation. The autoradiographic exposure time was 2-4 days. Scale bar, $10 \mu\text{m}$. *a*, Heavily labelled multiple loops at 8 units (ref. 16) on the heteromorphous long arm (HTA) of chromosome I. The level of labelling is very high over the whole length of the loop. *b*, Partially labelled loop pair at 54 units (ref. 16) on the HTA of chromosome I. There is a distinct gradient in the amount of silver grains, indicating that transcription proceeds in the direction shown (arrow). The unlabelled region can also be seen (open arrow).

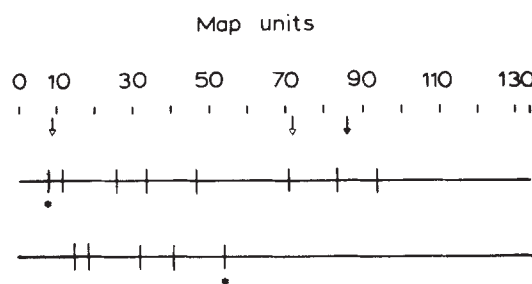


Fig. 3 Diagrammatic representation of the loops labelling on chromosome I of *T.c. carnifex* using pTcS1 to hybridise to the nascent RNA transcripts. The numbers associated with each loop give the distance from the left end of the chromosome in terms of units described by Callan and Lloyd¹⁶. The labelled loop pairs are drawn as bars on a line that represents the entire length of chromosome I. The centromere is at 86 units (arrow), and the heteromorphous region (HTA) lies between 9 and 72 units (open arrows). The top line represents the longer HTA, the bottom line the shorter HTA. The two loop pairs marked with asterisks are those at 8 units on the long HTA and at 54 units on the shorter HTA that are shown in Fig. 2. Conditions of hybridisation were as described in Fig. 2 legend.

that had been digested with *Bam*HI, electrophoresed on agarose and transferred to nitrocellulose filters¹⁰. Both clones hybridised to a ladder of sequences corresponding to monomers and multimers of a 330-base pair sequence (Fig. 1B), confirming that we had cloned a tandemly repeated sequence.

DNA from clone pTcS1 was then nick translated with 3 H-labelled triphosphates¹¹ and used as a probe in DNA/RNA transcript hybrids onto lampbrush loops from *T.c. carnifex*¹². After a 72-h autoradiographic exposure, several loops were heavily and specifically labelled. Most of these loops were situated on the long arm of chromosome I (Figs 2, 3). Results using the cloned satellite sequence confirm and extend results obtained using highly repeated DNA isolated by renaturation kinetics, by caesium salt centrifugation and by isolation of restriction fragments from agarose gels¹³. The level of labelling over some of the loops, and the short autoradiographic exposure needed, indicate that in some cases, the loop RNA consists almost exclusively of satellite DNA transcripts (Fig. 2a).

Ribonuclease treatment of lampbrush chromosome preparations (2 h at 37°C in a solution of $100 \mu\text{g ml}^{-1}$ ribonuclease A and $1,000 \text{ units ml}^{-1}$ ribonuclease T1 in $1 \times$ SSC) before incubation with labelled pTcS1 completely eliminated *in situ* hybridisation. No part of any lampbrush chromosome or any other structure became labelled in our autoradiographs, even after exposures of several weeks. Pretreatment of preparations with deoxyribonuclease 1 left the level of *in situ* hybridisation with pTcS1 unimpaired. Labelled, alkali-denatured pBR322 DNA was also used as a control, using precisely the *in situ* hybridisation and washing conditions as for pTcS1. Autoradiographs of pBR322 preparations were completely negative.

There are two important aspects to this study. The first is that we consider that our data provide the first clear evidence that DNA belonging to the satellite class is transcribed during the lampbrush stage of oogenesis in *T.c. carnifex*. The second is related to the unusual structure of the long arm of chromosome I in this species, and will be discussed later.

We consider it unlikely that the RNA transcribed from a satellite sequence will be translated. If it were, then any polypeptide produced would be very unusual. However, the question that remains is why satellites should be transcribed at all during the lampbrush stage of oogenesis. One possibility is that the RNA transcripts of satellite sequences in some way regulate the expression of other, possibly translatable, sequences in the germinal vesicle. Such control would be stage specific, and studies on this problem are in progress in our laboratory. A

second explanation might be that transcription of satellite sequences in the germinal vesicle is incidental, and that on a lampbrush loop the RNA polymerases overrun normal termination signals and read right through stretches of satellite DNA. The majority of the highly repeated DNA in *T.c. carnifex* is clustered in pericentric regions, and on the long arm of chromosome I (refs 7, 13, 14). If transcription does overrun normal termination signals, satellite DNA is more likely to be transcribed on chromosome I simply because there is so much of it in this region.

The presence of highly repeated DNA on the long arm of chromosome I correlates in an unknown way with structural heteromorphism and a lack of chiasma formation in this

chromosome arm in *Triturus cristatus*¹⁴⁻¹⁷. We have evidence that the distribution of the satellite sequences on the two long arms of chromosome I may be different¹³. We do not know why such a situation should exist, what evolutionary significance it may have, or why satellite DNA should be transcribed on this unusual chromosome.

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Oocytes in the testis

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In normal sexual differentiation, XX germ cells develop into oocytes in an ovary, and XY germ cells undergo spermatogenesis in a testis. In some genetic abnormalities of sexual differentiation, and other abnormal situations such as experimental mouse chimaeras, XX germ cells may be present in a male embryo^{1,2}. They will still migrate into the genital ridges, but in the environment of a testis they usually degenerate at or before the onset of meiosis. Whether they pursue a male or a female pathway of development before degeneration is not yet established. Very occasionally growing oocytes have been observed within seminiferous tubules, for example in a 16-yr-old hermaphrodite who was brought up as a girl and only came to medical attention because he wished to be recognised as a boy³. Such tubules usually form part of an ovotestis (refs 3-5 and P. S. Burgoyne, personal communication), though large numbers of primary and growing oocytes have also been observed in apparently normal testes of two mouse chimaeras, one an 8-d-old bilateral hermaphrodite (M. Spiegelman, personal communication) and the other a 5-d-old male⁶. The phenomenon is usually confined to very young animals, apart from one adult mouse chimaera (P. S. Burgoyne, personal communication) in which the tubules were disorganised and the oocytes that they contained were hyalinised, as though they had undergone atresia. In most cases (refs 3, 5, 6 and P. S. Burgoyne and M. Spiegelman, personal communications) the individuals were known to possess both an XX and an XY cell population, and the chromosomal sex of the oocytes was not established. In contrast, I report here that a substantial proportion of genetically sex-reversed male mice, in which all cells are initially XX in chromosome constitution, contained healthy growing oocytes within their testes up to at least 15 days of age.

The autosomal gene *Sex-reversed* (*Sxr*)⁷ causes genetically female (XX) mice to develop as phenotypic males. Such *Sxr*/+ XX males are sterile, as the XX germ cells are unable to undergo spermatogenesis. Apart from the absence of germ cells, the

testes are grossly normal in morphology. Cattanaach *et al.*⁷ examined some testes of sex-reversed mice in the prenatal and early postnatal period, and concluded that the XX germ cells, although present initially, are lost by 10 days of age.

During a further study of the testes of prepubertal *Sxr*/+ XX mice, I examined serial sections of 21 testes from sex-reversed mice, heterozygous for X-linked coat-colour markers and killed between 8 and 18 days of age. Six proved to contain growing oocytes. No oocytes were found in 30 testes from normal male sibs in the same age range. The fact that Cattanaach *et al.*⁷ did not detect any growing oocytes in their material is not in conflict with the present finding, for within this age range they only examined two animals (at 10 days of age).

Three of the sex-reversed testes contained one oocyte each, one contained two, one nine, and one contained 100 oocytes. The oocytes were located within the testis tubules (Fig. 1), usually in the neighbourhood of the rete testis (Fig. 1c). Each was surrounded by an incomplete layer of cells, morphologically similar to the Sertoli cells lining the tubules, though somewhat more flattened, and resembling in general appearance the incomplete layer of granulosa cells surrounding oocytes in the mouse ovary at birth. No sign was seen of the follicular growth that begins in the mouse ovary about 3 days after birth. The oocytes themselves, on the other hand, had enlarged considerably since birth, measuring 30-50 µm in diameter, about the same size as ovarian oocytes of the same age. Thus at this stage of development, oocyte growth and follicular growth are not necessarily associated. The state of preservation of the oocytes varied from good, with a well defined cell membrane, an intact nuclear membrane, and a distinct nucleolus, to very degenerate. In one case the nuclear material was represented by a large number (more than 100) of very small chromosome-like bodies (Fig. 1d), reminiscent of the highly condensed chromosomes seen in cells blocked in metaphase (see, for example, Paterson's illustration of air-dried preparations of dying embryos homozygous for the gene *oligosyndactyly*⁸).

Twelve adult (6-9 weeks) *Sxr*/+ XX testes were also serially sectioned, but none contained oocytes (0/12 compared with 6/21; *P* < 0.05, by Fisher's exact test). This suggests that all the oocytes would have degenerated before sexual maturity was reached. Perhaps such oocytes are unable to survive the changes in the testicular environment that must occur between 1 and 2 weeks after birth in the normal male mouse, to induce the entry of XY germ cells into meiosis.

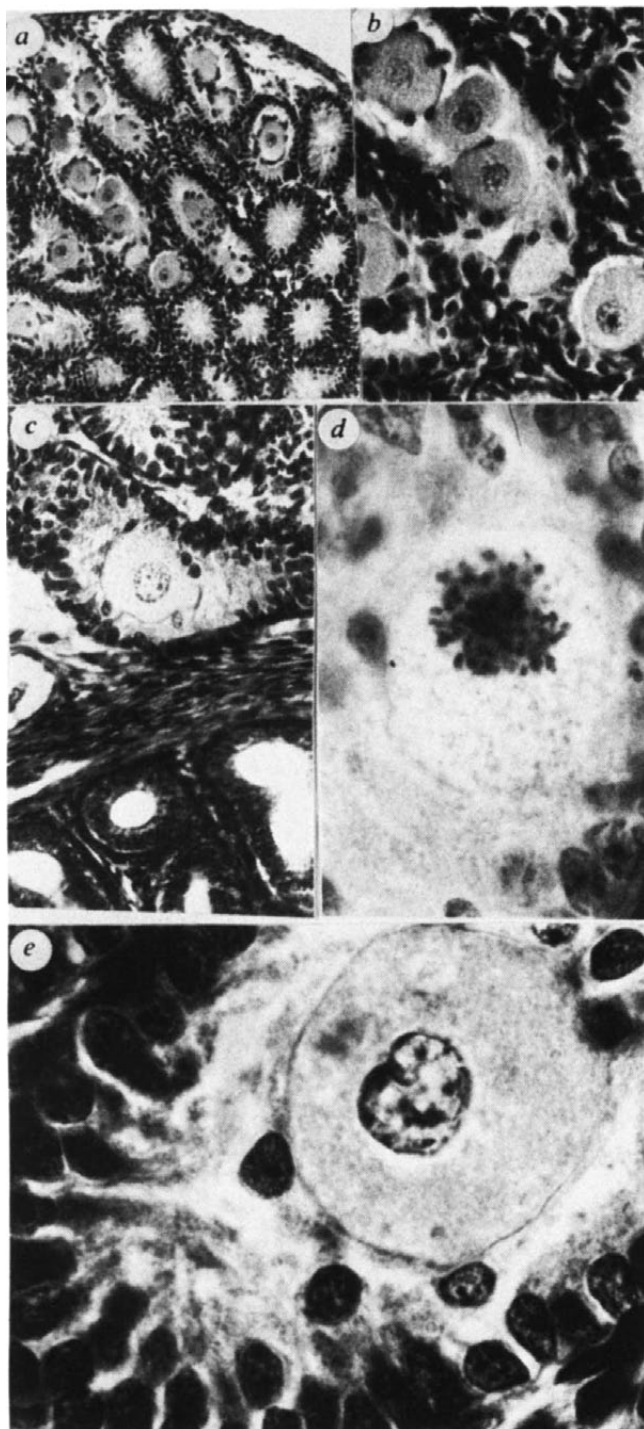


Fig. 1 *a*, Testis of 11-d-old XX *Sxr/+* male, showing numerous growing oocytes within the intact seminiferous tubules. *b*, Higher power view of some of the tubules shown in (*a*). The oocytes are surrounded by an incomplete layer of flattened follicle-like cells. *c*, Single oocyte in tubule of 10-d-old XX *Sxr/+* male, adjacent to rete testis. *d*, Degenerate oocyte, from 11-d-old XX *Sxr/+* testis. Nucleus appears to have broken down into numerous small chromosome-like bodies. *e*, Growing oocyte, with intact cell membrane and nucleus, from 15-d-old XX *Sxr/+* testis.

Does the fact that the XX germ cells in the sex-reversed testes were undergoing oogenesis, as befits their chromosomal sex, imply that they were developing autonomously? The testes of XX/XY mouse chimaeras contain initially both XX and XY germ cells. A study of such testes during the prenatal period⁹

suggested that XX but not XY germ cells entered meiotic prophase before birth, at the normal time for XX germ cells undergoing oogenesis in the ovary. XY germ cells can occasionally be induced to enter meiosis prematurely¹⁰, but apparently only if their environment is altered, for example, by transplantation beneath the kidney capsule¹¹, or explantation *in vitro*¹². In the rare case of an XX↔XY chimaera gonad differentiating as an ovary, XY germ cells have been reported to undergo oogenesis; one gave rise to a functional egg^{13,14}. XO germ cells, which resemble XY germ cells in having only one X chromosome, are capable of undergoing spermatogenesis if they are located in a testis, for example in an *Sxr/+* mouse, but the spermatozoa that they form are abnormal and show little or no motility^{7,15}. The few spermatogonia reported by Cattanaach *et al.*⁷ in the 2-d, 5-d and 6-d-old *Sxr/+* XX testes that they examined, might therefore have been XO in chromosome constitution, as were the spermatogenic cells isolated from the occasional tubule seen to be supporting active spermatogenesis in adult *Sxr/+* XX males¹⁵. Alternatively, if the spermatogonia in the newborn testes were XX, it would suggest that some XX germ cells can take a male developmental pathway, others a female pathway.

If this were so, development cannot be autonomous, but must be under some environmental influence. H-Y antigen, thought to have a male-determining role in the differentiation of the testis^{16,17}, seems more likely to act on the somatic tissues, inducing the formation of the testis tubules, than on the germ cells. The location of the oocytes in *Sxr/+* XX testes near the rete testis lends some support to the postulate of Byskov, that entry of germ cells into meiosis before birth is under the control of a diffusible meiosis-inducing substance (MIS)¹² produced by the mesonephric rete region in both female and male fetuses¹⁸. According to this postulate, germ cells in the testis would normally be protected from the effects of MIS by their location within the tubules, where a hypothetical meiosis-preventing substance, secreted perhaps by the Sertoli cells, would be present in high concentration¹⁹. The observations on *Sxr/+* XX germ cells reported here, as well as previous observations on XX↔XY chimaeras, would imply that germ cells with two X chromosomes are more susceptible to the meiosis-inducing influence of MIS than are germ cells with only one X chromosome, and hence are more likely to undergo oogenesis. Perhaps the germ cells that Cattanaach *et al.*⁷ described as spermatogonia in the newborn *Sxr/+* XX mice were those located further from the rete, that failed to come under the influence of MIS and hence started development along the male pathway.

I thank Dr B. M. Cattanaach for *Sxr/+* mice.

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Sequence-dependent variations in the backbone geometry of a synthetic DNA fibre

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There are many examples of proteins which interact with specific DNA sequences (see references cited in refs 1 and 2). It is not clear whether specificity is derived from proteins sensing directly the base sequence of the DNA or is a result of unique secondary or tertiary structural features induced by certain DNA sequences. Recent studies have indicated the occurrence of several types of symmetries and sequence irregularities in DNA regions which interact with regulatory proteins, but they do not provide information on three-dimensional structure³. The Watson-Crick base-paired double helix can be observed in different conformations⁴⁻⁶, depending on humidity and salt concentration. Within the limited resolution of X-ray fibre diffraction at high relative humidity and in the appropriate salt conditions, studies indicate that natural DNA as well as synthetic repeating polynucleotide duplexes adopt essentially the same average secondary structure, namely the B conformation⁷. This implies that all residues adopt a single backbone conformation. Recently, however, a high-resolution proton NMR study⁸ has demonstrated that a change from a regular conformation may occur at the junction region of block copolymers. ³¹P NMR studies strongly suggest that alternating polynucleotides can have alternating backbone conformations in solution^{9,10}. Klug *et al.*¹¹ have proposed an alternating structure for poly(dAdT)·poly(dAdT), based on a single crystal X-ray diffraction study of the tetranucleotide, dA-dT-dA-dT (ref. 12). We report here structural ³¹P NMR studies which show the existence of such variation in the fibre form of a synthetic DNA, poly(dAdT)·poly(dAdT). This confirms our earlier conclusion¹³ that natural DNA has a non-uniform backbone conformation.

Figure 1 shows an X-ray diffraction image from a poly(dAdT)·poly(dAdT) fibre at high relative humidity; the pattern is similar to the B form patterns reported by Davies and Baldwin¹⁴. From this image, the pitch and the rise per residue were found to be 32.3 and 3.3 Å, respectively, corresponding to 9.8 residues per turn. This implies that the alternating copolymer is in the B conformation in these conditions.

Figure 2 compares the ³¹P NMR spectra at 24.3 MHz from natural DNA fibres and poly(dAdT)·poly(dAdT) fibres at two different fibre orientations relative to the magnetic field, *H*₀. Observed NMR parameters are shown in Table 1. Strikingly, poly(dAdT)·poly(dAdT) fibres when orientated parallel to the magnetic field exhibit two well defined resonances, whereas natural DNA exhibits a single broad resonance in the same conditions (Fig. 2). The former result is consistent with the observation of a doublet in the ³¹P spectrum for a 145 base-pair poly(dAdT)·poly(dAdT) fragment in solution¹⁰. As the two resonances occur in nearly equal intensity, these results strongly imply an alternating conformation of the phosphodiester groups of DNA both in solution and in the solid state, stemming from the alternating base sequence of this polymer. As discussed previously¹³, the line width of the parallel spectrum is partly a result of imperfect orientation of the molecules relative to the fibre axis. We observed two resonances with a separation of 21 p.p.m. in the parallel spectrum for poly(dAdT)·poly(dAdT). The total line width is 39 p.p.m., of which instrumental factors and magnetic susceptibility may account for ≤5 p.p.m. Thus, the individual line width can be estimated to be about 13 p.p.m. (that is, 39 - 5 - 21 p.p.m.). This estimated value corresponds to that calculated for a 4-6 degree deviation of the helical axis

about the long axis of the fibre¹³. In other words, the observed individual line width for poly(dAdT)·poly(dAdT) can be explained by a small amount of molecular disorientation in the fibre. Applying similar reasoning to the natural DNA spectra, the distribution of chemical shifts extends over 24 p.p.m. (that is, 42 - 5 - 13 p.p.m.), which we take as a reflection of the limit of variation in the orientation of the phosphodiester group in DNA with heterogeneous sequences. The deviation in the orientation of the phosphodiester group in natural DNA was previously estimated to be of the order of ±20° from the average orientation about any of the individual orthogonal axes¹³. Note that the extremes of this range may be related to ribose ring puckering¹², but do not necessarily imply large changes either in base stacking conformations or in individual dihedral angles of the backbone. Thus, we conclude that irregularity in the backbone conformation is an ubiquitous phenomenon not restricted to a small region of DNA; presumably the irregularity is sequence-dependent. This irregularity of phosphodiester conformation is, of course, highly accentuated in poly(dAdT)·poly(dAdT). The high affinity of the *lac* repressor for this duplex¹⁵⁻¹⁸ may depend on just such structural characteristics.

The average chemical shift of the parallel spectrum for natural DNA occurs between the two resonances of poly(dAdT)·poly(dAdT). This implies that the conformations of the phosphate group in ApT and TpA sequences deviate in opposite senses from its average conformation in the B form of DNA. It is of interest that the one resonance, which is shifted downfield by 12 p.p.m. from the peak of the parallel spectrum of natural DNA (see Table I), occurs at the position of the maximum peak of the parallel spectrum for the A form of natural DNA¹³. This may suggest that the orientation of one of the phosphodiester groups is similar to its orientation in the A form of DNA. One plausible interpretation for the chemical shift changes is that the ionic group, O=P-O⁻, of the one of the

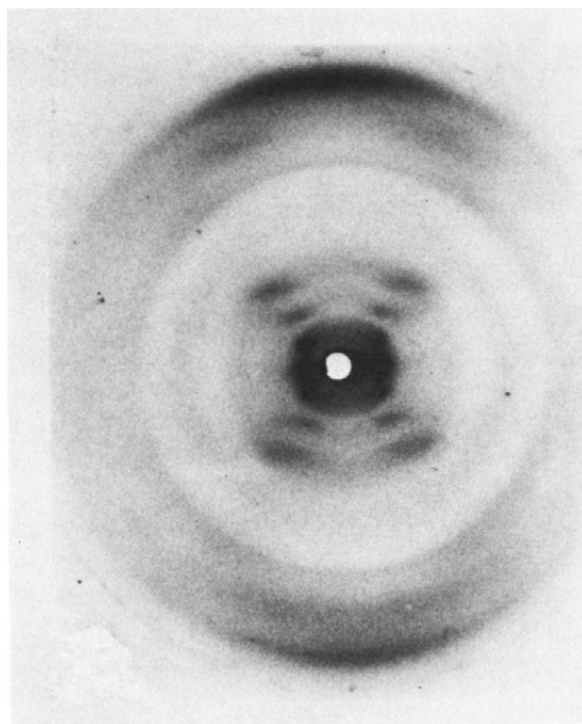


Fig. 1 X-ray diffraction photograph from a poly(dAdT)·poly(dAdT) fibre at room temperature and 98% relative humidity. The alternating copolymer (P. L. Biochemicals) was dissolved in a small amount of 10 mM NaCl, heated in a boiling water bath for about 1 min, and then cooled slowly to room temperature. The fibres were formed at 4 °C; otherwise their preparation and the collection of the diffraction pattern are as described previously²⁰.

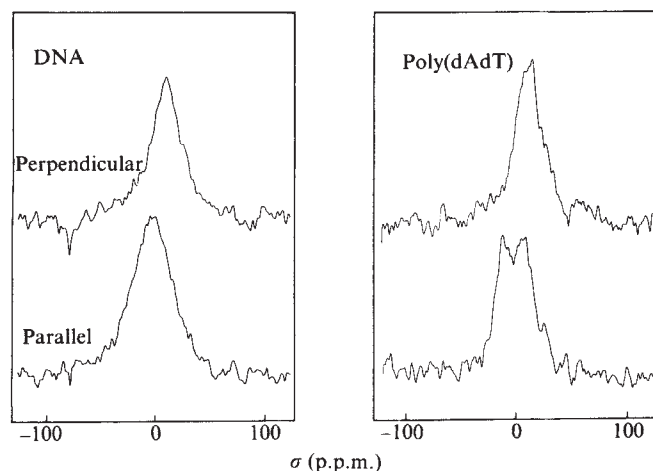


Fig. 2 The proton dipolar decoupled and cross-polarised ^{31}P NMR spectra (24.3 MHz) at 25 °C from poly(dAdT)-poly(dAdT) fibres (right) and salmon sperm DNA fibres (left). The top spectra for both DNAs were obtained from perpendicular-orientated fibres (relative to the magnetic field) and the bottom spectra from the parallel-orientated fibres. The method of controlling humidity and instrumentation were as described previously¹³. Measurement conditions; spectral window ± 20 kHz, data points 1,024 real, cross-polarisation time 1.5 ms, delay time 2 s, line broadening 50 Hz, scan numbers 21,000 for the DNA and 70,000–80,000 for the synthetic copolymer.

phosphates is pushed out on the surface of the duplex while the other is pushed into the major groove of the DNA, as judged from the general features shown by phosphate groups in the B and A form structures, respectively^{4,6,7}. Therefore, the characteristic cleavage of the P–O(3') bond of adenosine in poly(dAdT)-poly(dAdT) by pancreatic DNase I (ref. 19) may not actually be sequence-specific in a rigorous sense but rather the result of the difference in its accessibility to the enzyme. As a consequence, the upfield resonance is tentatively assigned to the phosphate of the ApT sequences and the downfield resonance to the phosphate in the TpA sequences.

Finally, we will consider the spectra obtained when fibres are orientated perpendicular to the applied magnetic field. To account for the perpendicular spectra, reorientational motion about the helical axis must be considered¹³. In principle, the perpendicular spectrum should display a doublet, assuming that the molecules are rigid and that all phosphodiester conformations are symmetrical with respect to the helical axis. The perpendicular spectrum for natural DNA exhibits a singlet (Fig. 2), which was interpreted previously in terms of rotational motion about the helical axis¹³. Furthermore, the line width of the parallel spectrum is broader by 10 p.p.m. than that of the

perpendicular spectrum for this DNA as a result of the variation in phosphodiester orientation. The same considerations may be applied to the alternating polynucleotide. A rather asymmetrical singlet was observed (Fig. 2), whereas a doublet would be expected even with rapid rotational motion about the helical axis. By using the estimated individual line width (13 p.p.m.) the separation of the resonances, if any, for the perpendicular spectrum would be about 10 p.p.m. or less, so that the doublet appears as a broad singlet at the frequency used here, but would possibly be split at higher frequencies. The structural considerations from the NMR data on the phosphodiester group in this synthetic polynucleotide will be discussed in more detail elsewhere.

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X-ray structure of the ternary complex Zn(II)-ATP-2,2'-bipyridyl and possible model for ATP transport

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Table 1 ^{31}P NMR parameters obtained at 24.3 MHz from salmon sperm DNA and poly(dAdT)-poly(dAdT) fibres at different orientations to the magnetic field

	RH (%)	Rotation angle (deg)*	Chemical shift† σ (p.p.m.)	Line width (p.p.m.)
Na-poly(dAdT)-poly(dAdT)	98	0	-17.1	4.0
		90	5–13	29
Na-DNA‡	98	0	-5	42
		90	8	32

* Rotation angle of zero corresponds to a parallel orientation of the fibres to the magnetic field and 90° to a perpendicular orientation.

† Measured from trimethylphosphate as external reference; upfield is positive.

‡ Data from ref. 13.

Ternary complexes between metal ions, nucleotides and aromatic heterocyclic amines are being investigated by X-ray diffraction as possible models for enzyme-metal-substrate interactions^{1,2}. Kennard and coworkers³ have reported the crystal structure of the ATP disodium salt. Although more recently several crystal structures of binary and ternary complexes between metal ions and nucleoside monophosphates have been reported, no structure combining metal complexes with nucleoside di- or triphosphates has been reported. Most divalent metal ions catalyse the nonenzymatic transfer of phosphate from nucleoside polyphosphates to various acceptors⁴. Therefore, these compounds in water solution undergo extensive hydrolysis in the presence of metal ions, leading to the formation of the monophosphates; for instance, at pH 6.5, Cu^{2+} accelerates the hydrolysis of ATP by a factor of about 300 (ref. 5). We report here the crystal and molecular structure of the complex $[\text{Zn}(\text{II})\text{-H}_2\text{ATP-2,2'-bipyridyl}]_2\cdot 4\text{H}_2\text{O}$, determined by single crystal X-ray analysis. The complex molecule provides a possible model for ATP transport and phosphate group transfer mechanism.

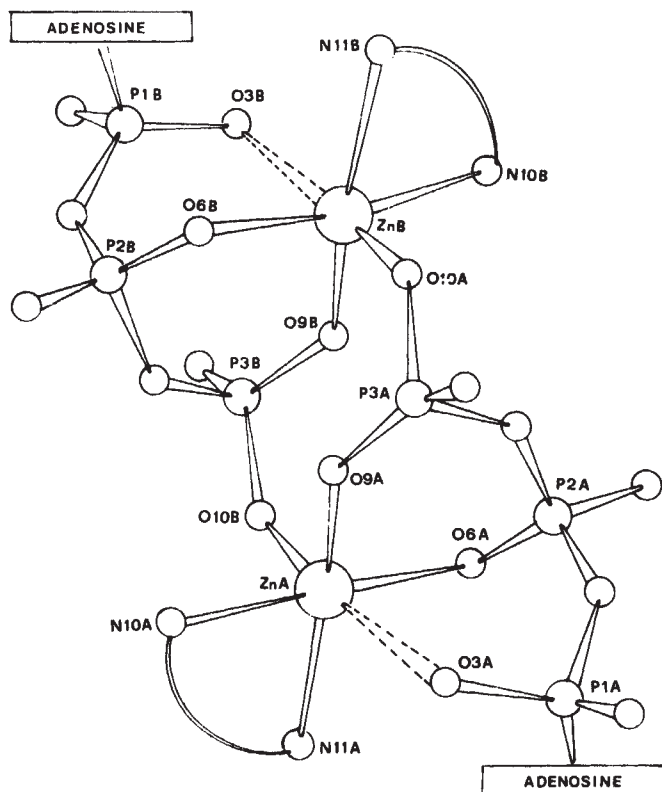


Fig. 1 Schematic drawing of the molecule, showing the mode of bonding of ATP.

Sigel and coworkers have shown that the presence of a second ligand possessing stacking capabilities (bipyridyl, phenantroline, tryptophan) greatly reduces the metal ion promoted dephosphorylation⁶. The inactivity towards hydrolysis is due to the formation of very stable ternary complexes between the nucleoside triphosphate, the metal ion and the second ligand. Stacking interactions between the heterocyclic base and the

purine or pyrimidine moieties enhance the stability of the ternary complex⁷.

We have been able to isolate microcrystalline compounds between ATP, 2,2'-bipyridyl and some 3d metal ions, such as Mn(II), Co(II), Cu(II), and Zn(II) in 1:1:1 ratio. These compounds are readily formed on mixing hydroalcoholic solution of $\text{Na}_2\text{H}_2\text{ATP}$ and 2,2'-bipyridyl with an aqueous solution of the metal sulphates in equimolar ratio. On the basis of the analytical and X-ray results, the complexes can be formulated as $[\text{M}-\text{H}_2\text{ATP}-2,2'\text{-bipyridyl}]_2 \cdot 4\text{H}_2\text{O}$. The solid compounds formed are essentially insoluble in most common solvents and only slightly soluble in water. Their X-ray powder patterns are very similar and only slightly soluble in water. Their X-ray powder patterns are very similar, minor differences being noted for the copper complex. Very small crystals, only for the zinc compound, were obtained on mixing dilute solutions of the reactants at pH 4. Trials to grow larger crystals failed, owing to the precipitation of hydrolysis products upon the crystals on standing.

X-ray diffractometer data were collected on one of these crystals with the following results: $a = 11.105(3)$, $b = 25.223(7)$, $c = 10.539(3)$ Å, $\beta = 91.34(4)^\circ$, monoclinic, space group $\text{P}2_1$, with two dimeric units in the cell. Patterson and Fourier syntheses gave readily all the atomic positions, which were subsequently refined by least squares methods to an R factor of 9.8% over 1,617 reflections with intensity greater than twice their standard deviation.

The structure of the compound consists of dimeric molecules in which two zinc atoms are held together by two $-\text{OPO}-$ bridges from the γ -phosphate groups of two ATP molecules, an arrangement which leads to the formation of an eight-membered puckered ring (Fig. 1). In the dimer both zinc atoms show a distorted octahedral coordination, formed by two oxygen atoms from different γ -phosphate groups, one oxygen atom from the β -phosphate group and the two nitrogen atoms of the bipyridyl ligand. The sixth position is completed by an α -phosphate oxygen atom which is only weakly bound, the $\text{ZnA}-\text{O3A}$ and $\text{ZnB}-\text{O3B}$ distances being respectively 2.70(4) and 2.42(4) Å. The other Zn-O distances average 2.02(3) Å and the mean Zn-N distance is 2.15(4) Å.

The structure is held together by strong intermolecular stacking interactions between bipyridyl and purine groups, and by

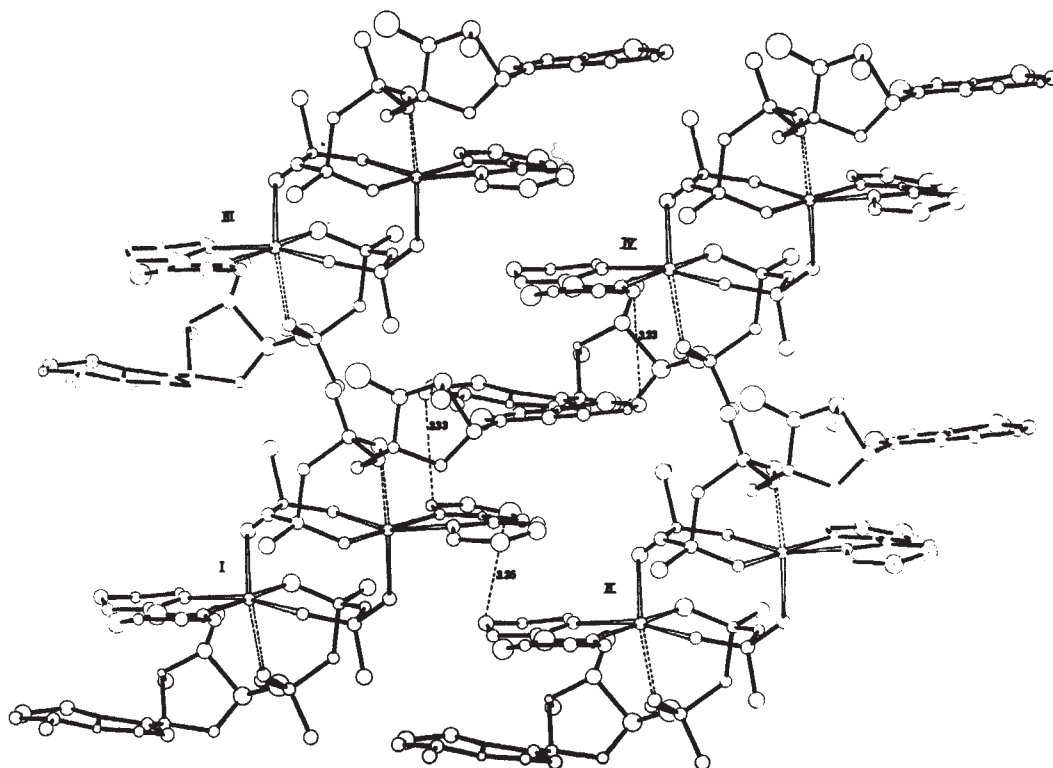


Fig. 2 Diagram of four dimeric units in $[\text{Zn}(\text{II})-\text{H}_2\text{ATP}-2,2'\text{-bipyridyl}]_2 \cdot 4\text{H}_2\text{O}$ showing the intermolecular stacking. Water molecules have been omitted for clarity. The molecules are in the following positions: I (X, Y, Z); II ($X-1, Y, Z$); III ($X, Y, Z+1$); IV ($X-1, Y, Z+1$). There are two types of stacking interactions: a bipyridyl-purine interaction and a bipyridyl-bipyridyl self stacking. The stacking sequence is the following (B, bipyridyl; P, purine): $-B_b(\text{II})-B_a(\text{I})-P_b(\text{IV})$; $P_a(\text{I})-B_b(\text{IV})-B_a(\text{III})-$; and so forth (a, b refer to the two monomers in the same dimeric molecules). The shortest distances in the stacking sequence are also shown.

hydrogen bonds between most of the polar groups. Water molecules appear to be disordered in the lattice.

Although one must be careful in drawing conclusions from the solid state, the present structure is in agreement with the results from the solution studies concerning the stability of ATP ternary complexes towards hydrolysis⁷: (1) the chelating bipyridyl does not allow interaction of the metal ion with N7 of the base moiety; (2) binding of the phosphate chain takes place essentially through the β - and γ -phosphate groups, the interaction with the α -phosphate being rather weak. Another feature arising from the solution studies is the stability enhancement due to intramolecular stacking between purine and bipyridyl. The present structure shows very strong stacking interactions between different molecules, as expected in a crystal, where intermolecular forces are much stronger than in solution (Fig. 2).

Finally it should be pointed out that the present structure also supports the mechanism proposed for the enzyme-catalysed transfer of the phosphoryl group from ATP⁷. In fact, shift of the metal ion along the phosphate backbone into β , α coordination leading to a labile γ -phosphate group, can be simply performed by a slight shortening of the Zn–O3 bond and consequent loosening of the Zn–O9 bond.

Bond lengths and angles and detailed conformational analysis of the molecule as well as physicochemical characterisation of all the four complexes will be reported elsewhere.

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Sequence of a ribosomal RNA gene intron from *Tetrahymena*

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Recently, several genes coding for messenger RNA, transfer RNA and ribosomal RNA in eukaryotes have been found to be interrupted in their coding regions by DNA sequences which are not represented in the mature RNA transcripts (see ref. 1 for review). Many of these intervening sequences, or introns², are now known to be transcribed in the precursor RNA, from which they are subsequently processed out to form the mature RNA^{3–10}. As the intron–exon junctions must in some way be recognised for accurate splicing, the nucleotide sequences of these regions from a number of protein-coding and tRNA genes have been analysed^{11–18}. Sequence homologies were found at the splice points of the protein-coding gene introns from diverse organisms^{12,13}, but the tRNA intron boundaries were not similar to these^{14,15}. This has led to the speculation that different splicing activities are necessary for the processing of introns in mRNA and tRNA precursors¹⁹. We report here the sequence of a ribosomal RNA gene intron from *Tetrahymena* in which intron–exon junctions differ from those analysed to date.

In *Tetrahymena pigmentosa*, restriction endonuclease mapping and electron microscopic analysis of R-loops²⁰, as well as recent S₁ nuclease mapping experiments (ref. 21 and J. Engberg, personal communication) have demonstrated that an intervening sequence is present in the macronuclear 25S ribosomal RNA genes of certain strains, but is absent from the rDNA in others. All the rDNA molecules of each strain are of one kind, either containing or lacking the intron, and intron-containing genes must therefore be functional. Recently, evidence from R-loop hybridisation has been used to show that such intervening sequences are indeed transcribed in the closely related species *Tetrahymena thermophila*^{7,10}. Thus, the DNA sequences at the boundaries of the intervening element correspond to points at which the precursor rRNA must be spliced.

To study the intervening sequence in *T. pigmentosa* rDNA in more detail, the recombinant plasmids pTp6001 and pTp8002 were constructed from the vector pBR322, as described previously²⁰. Briefly, pTp6001 contains a 1.6-kilobase *Hind*III fragment from the rDNA of strain 6UM, which includes a part of the 25S rDNA and the intron that interrupts it. Plasmid pTp8002 contains a 1.2-kilobase *Hind*III fragment that includes only the corresponding 25S rDNA of strain 8ALP, and lacks the intron. Restriction fragments from these plasmids were sequenced by the chemical cleavage method of Maxam and Gilbert²². Position and orientation of the 5'-labelled strands used are indicated in Fig. 1. The entire sequence of the 407-base pair intron and the surrounding rDNA is depicted in Fig. 2, which is a composite of the sequence data obtained from both pTp6001 and pTp8002. In those regions where both DNAs have been analysed (see Fig. 1), the nucleotide sequences are identical.

By directly comparing the sequence from pTp6001 with that from pTp8002, a distinct position is found at which the intron interrupts the coding region (Fig. 3). This is in contrast to all other intron–exon boundaries sequenced to date, in which the exact point of interruption is ambiguous due to the presence of directly repeated nucleotides at the junctions^{12,13}. In precursor RNA from protein-coding genes containing introns, the position of the splice within the repeats has been proposed to occur such that all introns begin with 5'-GU and end with AG-3' (ref. 13). The ends of the *T. pigmentosa* rDNA intron do not obey this rule, and furthermore, are completely different from recent model sequences proposed for mRNA-type intron–exon junctions^{1,13,24}. In addition, these rRNA splice points bear no resemblance to the sequences from yeast tRNA intron boundaries^{14,15}. At the *T. pigmentosa* rDNA intron junctions themselves, we note the presence of the sequence TCX|XAA at both the 5' and 3' ends; whether or not this has a role in defining the splice point in the transcript is unknown.

For recognition and accurate processing of an intron by a splicing mechanism, more information is necessary than is found in the short regions of nucleotide homology seen at mRNA intron boundaries. It is therefore likely that secondary structure of intron-containing precursor RNAs is also important for recognition of the regions where splicing is to occur. In this context, it is interesting that the *T. pigmentosa* rDNA intron itself is fairly rich in potential secondary structure, as determined by computer analysis and indicated in Fig. 2. We also note that both ends of the intervening sequence are rich in AT.

Recently, Allet and Rochaix²³ have determined the sequence of the boundaries of the intron in the *Chlamydomonas* chloroplast 23S ribosomal RNA gene. In this case, too, no similarity to either the mRNA or the tRNA intron junctions of nuclear genes is present. As we have found in *T. pigmentosa* rDNA, the ends of the *Chlamydomonas* rDNA intron are unequivocally defined. Although some sequence homology between these two rDNA introns can be found, any speculations on a model sequence for rRNA intron–exon junctions must await the analysis and comparison of other intron-containing nuclear and organellar rDNAs. If, however, the differences in the splice point sequences of tRNA and mRNA introns reflect a difference in

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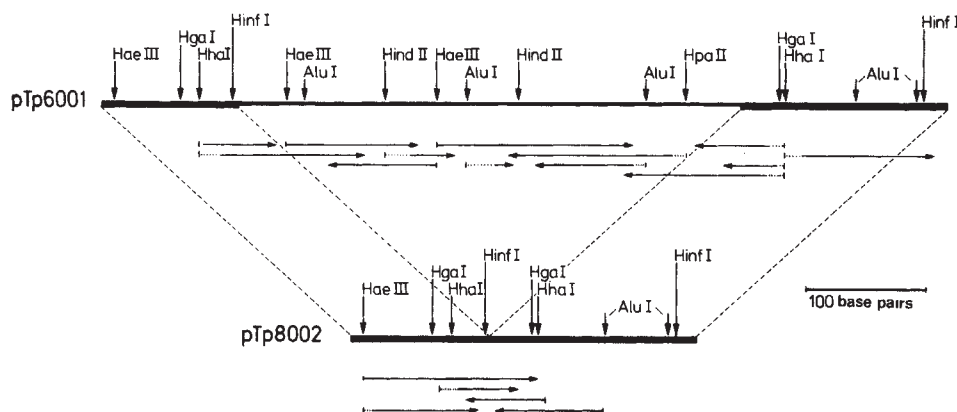


Fig. 1 Partial maps of clone pTp6001, which contains a rDNA intron, and clone pTp8002, which lacks this intron. The connecting lines show the regions of homology between these two clones. All restriction sites are given for those enzymes which were used to prepare fragments for sequencing. Horizontal arrows below each map indicate the position, direction and length of sequence determined for each fragment. After 5'-end-labelling with [γ - 32 P]ATP and T4 polynucleotide kinase, fragments were strand separated in 0.1 M KOH and 1 mM EDTA, or were digested with a second restriction enzyme, and

were then subjected to electrophoresis on 6% or 10% acrylamide gels. DNA sequencing was carried out using the chemical cleavage technique of Maxam and Gilbert²² with minor modification. The degraded strands were separated on 10%, 20% or 24% acrylamide, 7 M urea gels ($2.5 \times 200 \times 400$ mm). After autoradiography, the nucleotide sequences derived from each fragment were stored by computer and analysed for overlaps with each other.

5' GATGACCAATGTGATTCTGCCAGTCTCTGAATGTCAAAGTGACGCAATTCAACCAAGCGGGTAAACGGCGGGAGTAAGTCTCTAAATT
GCAAAATTACCTTTGGAGGAAAAGTTATCAGGCTCTGACCTGATAGCTAGCTTTAAACCAATAGATTGCATCGTTTAAAGCAAGCGCTCAAAT
TGCGGAAAAGGTTCAACAGCGCTTCAGTACCAAGTCTCAGGGAACTTTGAGATGGCTTGAAGGATATGGTAATAGCTGACGACAGGGTCTTA
ACCAACGACGCAAGTCTAAGTCAACATTTGGTGTGATATGGATGACGTTTACAGACTAAATGTCGGTGGGGAAGATAGGTATTCTTCTCATAAGA
TATAGTCGAGCTCTCTTAATGGGAGCTGGCGGATGAAGTGGTGAACACCAAGAGCGCGGGGAAGTAAATAAACATAATATTAGTTTGGACTAAT
CGTAAGGTAGCCAAATGCTCGTCTCTAATAGTGAAGCGCATGAATGATTAATGAGATTACCACTGTCTCTACTATCTAGCGAACCCAGCT
AAGGGAACGGGCTTAGAATAATCAGCGGGGAAGAGACCTGTTGAGCTTGACTCAGTCTAACT 3'

Fig. 2 Sequence of the 407-base pair rDNA intron and adjacent regions. The sequence presented is a composite of both clones pTp6001 and pTp8002. Only the strand which corresponds to the ribosomal RNA is shown. The intron-exon transition points are indicated by arrowheads; exon regions are underlined. About 100 base pairs of the exon regions were analysed for both clones and were found to be identical. The recognition sites of restriction endonucleases used in the sequence analysis are overlined: AGCT (AluI), GGCC (HaeIII), GACGC (HgaI), GCGC (HhaI), GTCAAC (HindII), GACTC (HinfI), CCGG (HpaII). The sequence

was screened for potential secondary structure by a computer search for inverted repeat sequences. Only those inverted repeats which are less than 15 nucleotides apart from each other, such that they might form hairpins, are indicated by head-to-head arrows. The inclusion of G-T base pairs, in addition to G-C and A-T base pairs, has been allowed in the inverted repeats. Computer programs used were developed by G. Osterburg and R.S. (unpublished).

their splicing mechanisms, we suggest that at least one more splicing activity exists which is necessary for the processing of intron-containing ribosomal RNA precursors.

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pTp6001 5' ATGACTCTCTAATTGAAA 3' 5'-End of intron
pTp8002 ATGACTCTCTTAAGGTAGCC
pTp6001 GGACTAATCGTAAGGTAGCC 3'-End of intron

Fig. 3 Sequence at the 5' and 3' intron-exon junctions in clone pTp6001 compared with the uninterrupted sequence of clone pTp8002. The unequivocal transition points between intron and exon are indicated by arrowheads. Exons are underlined.

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MATTERS ARISING

Problems with mesospheric electric field measurements

HALE AND CROSKY¹ have recently reported some unusual results regarding the mesospheric fair weather electric field. These results are extremely interesting for two reasons: the reported values of the electric field are much larger at all altitudes than those previously reported, and the field is observed to increase dramatically at ~65 km altitude. There are several reasons why we feel that the reported data should not be interpreted in the straightforward way proposed by Hale and Croskey, but the possibility that their result is correct certainly justifies considerable further work in this area.

We have performed similar experiments, two of which have been reported². We drew two conclusions from our results. First, the experiment is an extremely difficult one, with large anomalous charging events of both sign seriously and frequently perturbing the potential of the central payload with respect to the medium as determined by the common mode variation of several ≈ 1 m long antennas. Comparison of these results with unpublished night-time results suggests that the photoelectric effect is not responsible for the observed potentials.

On the basis of body-mounted Gerdien condenser data, Hale and Croskey assert that the potential of their payload did not vary significantly during flight. However, a Gerdien condenser only measures the potential of the payload with respect to the gas passing through the condenser. Essentially all measurements that have been made with extended antennas of the potential of moving vehicles in the 20–80 km altitude range show large, rapidly varying potentials (ref. 2 and personal communications from F. S. Mozer, R. H. Holzworth, and L. C. Hale). On the other hand, Gerdien condenser payload potential measurements seem to show this sort of effect relatively seldom^{1,3}. These contrasting results strongly suggest that the potentials seen by the long antenna measurements are being developed sufficiently far from the payload to fool the Gerdien condenser. Gas or ion entrainment effects inside the Mach cone may also be confusing the issue. The fact that large detached potential drops^{4–8} and ion entrainment effects⁹ have been observed in other admittedly disparate experimental circumstances suggests that similar effects are possible in this case. Regardless of the actual mechanism responsible, a rigorous, physically detailed explanation of the discrepancy between probe and condenser measurements of payload potentials in the mesosphere must be presented by Hale and Croskey (or others)

before their measurements can be believed. Cryptic allusions to accumulated experimental experience are no substitute for sound physical argument.

Second, we detected an electric field of 20 mV m⁻¹ at an altitude of ~70 km in fair weather, during magnetic quiet, at White Sands, New Mexico, a low latitude site. This measurement was made during a period when the central payload was not anomalously charged. Our experiment and the Hale and Croskey experiment used identical input amplifiers, and were very similar in geometry. Taken together, the measurements imply that Hale's hypothetical global mesospheric circuit must, in fact, be confined to high latitudes or the nightside, or both. This conclusion, and Hale's conclusion that the mesospheric circuit has 'holes' opened in it by auroral activity, has serious observable consequences. The horizontal fringing fields produced at the edges of the 'holes' and the southern edge or the terminator edge of the circuit would be observable at balloon altitudes. Hale *et al.*³ suggested that the critical observations have been suppressed because balloon observers tend to attribute all data taken during periods of inverted vertical fields to electrified clouds. However, for the case reported by Hale and Croskey, these horizontal fields would not be accompanied by an inversion of the vertical field. Hundreds of hours of balloon data have been taken under circumstances where the fringing fields should have been seen, and they have not been observed (personal communications from F. S. Mozer, and R. H. Holzworth)^{3,4}. More important, good agreement is regularly reported between balloon and ionospheric measurements of the horizontal electric field^{10,11}. This agreement argues strongly against the existence of these fringing fields, and hence of the hypothetical mesospheric circuit.

A final criticism of both the Hale and Croskey results and the Bragin *et al.*¹² results concerns vehicle dynamics. The large field peaks reported by both groups occur when the payload is travelling at a velocity greater than or equal to Mach 1, and is passing through the region of maximum aerodynamic deceleration. In this context, anomalous 'field values' are not remarkable. What is remarkable is that there are any non-anomalous results from this region.

We would like to stress that there are good physical reasons for knowing the mesospheric electric field^{1,2} and the properties of the mesosphere. The points of controversy raised here cannot be resolved without extensive further work, involving parallel approaches, and experiments with controls and comparisons.

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HALE REPLIES—Although Bering, Benbrook, and Sheldon do not only respond to our letter but also to statements made elsewhere, they have, nevertheless, raised questions that need to be resolved before problems of electrical coupling through the atmosphere can be solved.

We described a quiet-to-disturbed enhancement in the lower atmosphere vertical electric field accompanied by a decrease in a large mesospheric field. The idea of a 'global' mesospheric circuit, possibly related to an aerosol layer, was one suggestion made to explain this effect and the USSR data¹.

We have performed additional measurements which show other aurorally related effects, including field reversals (suggestive of earlier observations by Freier² and by Olson³) and large amplitude (~100%) wave-like structure in the lower atmosphere field (L.C.H., C. L. Croskey, and D. F. Young, in preparation). These effects cannot obviously be explained by 'classical' theory, by 'mapping' of magnetospheric fields, or by a simple constant altitude 'global' mesospheric circuit. A mid-latitude sunrise measurement (16 September 1979, 05.30–07.18 LT, Wallops Island, Virginia) showed a relatively low and featureless electric field below 65 km, increasing to about 1.5 V m⁻¹ downwards at 20 km, the value measured by Bering *et al.*⁴. The obvious absence of 'anomalous charging' effects on this measurement, where the vehicle potential was determined by a simple 'blunt' probe⁵ in the front surface, gives us confidence that our high latitude results are real, as the vehicle velocity profiles were similar.

Thin linear electrodes parallel to the flow would seem to be superior to plates or spheres for electric field measurements when dominated by a continuum flow. A wire or rod of length L can be shown to have about the same conductance to the medium as a sphere of diameter L but will have far less photo-emission or turbulent wake effects. Plate electrodes would be sensitive to angle-of-attack variations.

Only a small fraction of the "hundreds of hours of balloon data" cited by Bering *et al.* were accompanied by simultaneous measurements of the auroral electric field, and when they were the agreement was not always good even during fair weather⁶. The limited extent of 'fringing fields' of even a non-global mesospheric circuit would be such that I doubt whether there is sufficient data to reject such a possibility out-of-hand, although this is not necessarily the only coupling mechanism. The local effects of aurorally produced charge separation, suggested by Freier², would apparently be capable of explaining some of the observations, particularly the reversals.

I echo Bering *et al.*'s plea for the acquisition of more data 'with controls and comparisons'. Perhaps this can be done by the international community during the forthcoming Middle Atmosphere Program (MAP). The several hundred meteorological class rockets necessary for a reasonable global description of the electric field in a variety of geophysical conditions is a minute effort compared to the tens of thousands of rockets that have been launched to acquire mesospheric wind and temperature data. These measurements should certainly be accompanied by balloon measurements but note that our coupling effect would not have been detected at intermediate altitudes.

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GABAergic mechanisms in the substantia nigra

MARTIN AND HAUBRICH¹ have confirmed the original reports^{2,3} of contralateral rotational behaviour following unilateral injections of the γ -aminobutyric acid (GABA) agonist muscimol into the zona reticulata of the

rat substantia nigra. They have also confirmed a previous report⁴ that intranigral perfusion of muscimol increases ³H-dopamine (DA) efflux in striatal perfusates loaded with radiolabelled DA precursor. We believe their conclusion, that contraversive rotation to intranigral muscimol is mediated via activation of nigrostriatal DA neurones, to be erroneous.

Unilateral intranigral injections of muscimol (100 ng μl^{-1} saline) were made² into the substantia nigra of unlesioned rats or of rats in which the ipsilateral nigrostriatal DA system had been destroyed 21 days previously by intracerebral injection of 6-hydroxydopamine (6-OHDA 8 μg per 4 μl) into the medial forebrain bundle⁵. Rotational behaviour was quantified in automated rotometers⁵ for 45 min and animals then taken for neurochemical analyses. 6-OHDA-lesioned rats with 90% destruction of the nigrostriatal DA system, as indexed by striatal tyrosine hydroxylase activity, exhibited vigorous contralateral rotation to intranigral muscimol that was indistinguishable from that shown by non-lesioned rats (Fig. 1). We therefore conclude that these circling responses are independent of dopaminergic mechanisms. Note that there is already evidence that nigral non-dopaminergic neurones control postural asymmetry⁶, and that Martin *et al.*⁷ were unable to demonstrate consistent antagonism of muscimol-induced turning by haloperidol. We find doses of haloperidol that are clearly blocking striatal DA receptors to have either very weak or no effect on rotational responses after intranigral administration of GABAergic drugs⁸.

In unlesioned rats, striata ipsilateral to intranigral injections of 100 ng muscimol showed⁸ elevated concentrations of DA (+98%, $P < 0.02$) and of the intraneuronal⁹ metabolite dihydroxyphenylacetic acid (+71%, $P < 0.01$) but not the extraneuronal metabolite⁹ homovanillic acid (+4%, NS). This neurochemical profile is consistent with that of the increased synthesis and decreased release of DA which follows inhibition of impulse flow in nigrostriatal DA neurones¹⁰, and therefore with a GABAergic inhibition of activity of nigral neurones¹¹. The finding by Martin and Haubrich¹ of elevated ³H-DA in striatal perfusates following intranigral muscimol could in part reflect the elevated synthesis of DA known to occur after inhibition of impulse flow in nigrostriatal DA neurones¹⁰.

Rotation after intranigral muscimol is most enduring and rats taken for neurochemical studies at 45 min after such treatment were rotating vigorously at this time. The resultant striatal neurochemical profile was consistent with reduced dopaminergic activity. In their reply Martin and Haubrich concede that DA release may be reduced at 45 min after

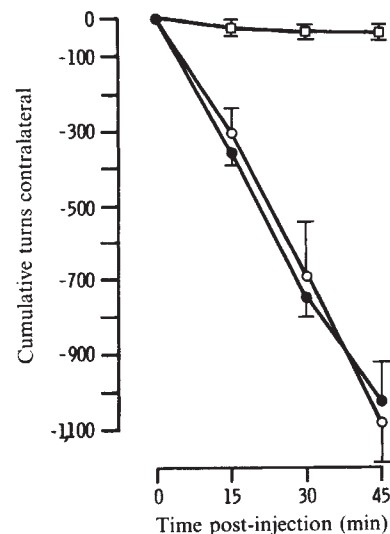


Fig. 1 Rotational responses to unilateral intranigral injection of 100 ng muscimol in male Sprague-Dawley rats with (○, $n = 5$) and without (●, $n = 10$) destruction of the ipsilateral nigrostriatal DA pathway induced with 6-OHDA. Intranigral injections were made under light ether anaesthesia as previously described², in 1 μl saline. 6-OHDA (8 μg per 4 μl saline ascorbate) was injected 21 days previously into the medial forebrain bundle⁵. Control animals received 1 μl saline into the nigra after 6-OHDA lesions (□, $n = 5$); such injections made into the nigra of unlesioned animals also induced negligible circling². Rotational behaviour was quantified in automatic rotometers⁵.

intranigral application of muscimol, when rats would still be rotating. This is inconsistent with their proposition that striatal DA release mediates the contralateral rotational response to intranigral muscimol. We have recently shown¹² that destruction of striatal cell bodies and striatal efferents by intrastriatal injections of kainic acid fails to attenuate turning following intranigral muscimol. Therefore striatal DA release cannot mediate this response.

Thus our behavioural findings suggest that contralateral rotation following intranigral injection of GABAergic drugs is not due to activation of the nigrostriatal dopamine system and our neurochemical findings suggest that the activity of this system can be inhibited by this procedure¹³.

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MARTIN AND HAUBRICH REPLY—We have reported that the efflux of ^3H -dopamine (DA) from the ipsilateral striatum increases during contraversive turning evoked by the intranigral infusion of muscimol in the rat. From these data we suggested that the release of striatal DA may mediate the muscimol-evoked contralateral turning¹. In a separate study using immobilised cats, Cheramy and co-workers have also demonstrated that the intranigral infusion of muscimol elicits a similar efflux of ^3H -DA from the ipsilateral striatum^{2,3}. The results from both studies indicate that dopamine release occurs in the ipsilateral striatum during the intranigral infusion of muscimol. We feel that our interpretation of the push-pull data are correct.

Waddington has challenged our suggestion that the release of DA might mediate muscimol-induced turning. This finding is based on two of his observations. First, the destruction of 90% of the striatal DA neurones with microinjection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle did not attenuate the turning evoked by the application of muscimol into the substantia nigra. Although the seeming complete lack of diminution in muscimol-evoked turning in 6-OHDA-treated animals is somewhat surprising, it does not preclude the possibility that the remaining 10% of the DA neurones might mediate the turning, especially in view of the supersensitivity which develops in postsynaptic DA receptors after 6-OHDA treatment⁴. Therefore, this negative finding does not incontrovertibly exclude the possibility that muscimol causes turning by stimulating DA release. Waddington has apparently not determined whether or not DA release occurs in the striatum of the 6-OHDA-lesioned rats following the intranigral microinjection of muscimol, nor whether haloperidol depresses significantly the muscimol-induced turning in 6-OHDA-lesioned rats as in intact animals⁵⁻⁷, and therefore these data are not conclusive in ruling out the role of dopamine release in the turning activity evoked by intranigral muscimol.

Waddington also suggests that muscimol inhibits the release of DA, whereas we have reported an enhanced DA release. Our conclusion is based on direct measurement of DA release from dopaminergic neurones during the time of the muscimol infusion, whereas those of Waddington are based on indirect evidence obtained from measurement of levels of DA and metabolites in tissue dissected 45 min after the microinjection

of muscimol. We feel, therefore, that there is no basis for comparing the two dissimilar pieces of information and that to debate the point is meaningless. We feel strongly that we are measuring DA release.

As haloperidol only partially antagonised the muscimol-evoked turning in another study⁵, we postulated the possible activation of at least one other neuronal system, perhaps GABA-like, in the activation of the muscimol-evoked turning. We did not wish to imply that DA release is the sole mediator of the turning. Contrary to Waddington's assertion, however, we feel that our evidence is quite compelling in indicating that striatal DA release, at least in part, may mediate the turning induced by intranigral muscimol.

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Is corticotropin releasing factor modulated vasopressin?

A RECENT report by Gillies and Lowry concludes that corticotropin releasing factor (CRF) is arginine vasopressin (AVP) modulated by other hypothalamic factors¹. This claim is based on several experimental observations. First, fractionation of a crude extract of stalk median eminence (SME) showed that three factors were required for full biological activity. Second, on Biogel P2, the major peak of CRF activity eluted in the expected position for AVP. Third, removal of AVP by cross-reacting the SME extract with vasopressin antisera dramatically reduced the CRF activity. Finally, SME extract from Brattleboro rats, which lack AVP genetically, contains 20% of normal CRF activity.

Our laboratory has also published evidence that the CRF activity of SME extract contains at least two factors which are required for full CRF activity². Furthermore, on the basis of studies of the CRF-like activity of peptides related to oxytocin and vasopressin, we showed that the greatest CRF-like activity was displayed by AVP, in which 40 ng contained 27% of the CRF-activity of one median eminence³. However, because we ob-

served normal CRF-like activity in a SME extract of DI-HOMO (Brattleboro) rats, we discarded the notion that AVP is the physiological CRF. On the basis of this evidence, we have proposed previously that the structure of CRF may closely resemble that of AVP.

Because our data comparing the CRF-like activity of Brattleboro SME extract with that of normal rats have not yet been published, we repeated these experiments in our laboratory. We used a semi-automated *in vitro* bioassay for CRF in which the ACTH released from normal rat pituitary halves is determined by measuring the corticosterone secreted by normal rat adrenal quarters in an automated superfusion system³. This bioassay has a linear dose-response relationship at 0.05-2.0 median eminence equivalents.

Median eminence extract from normal Long-Evans, normal Sprague-Dawley and DI-HOMO Brattleboro rats was prepared in 0.1 M HCl at a concentration of 25 μl per SME⁴. The test dose in all experiments was 20 μl , 0.8 SME equivalents, which was well within the linear portion of the dose-response curve for this bioassay³. After a 2-h preincubation, one set of each pair of pituitaries was incubated for 1 h with 20 μl of SME extract from Sprague-Dawley rats; the second set of each pair of pituitaries was incubated in buffer alone to serve as a control. After a second preincubation of 1 h, the test sample was incubated with one set of each pair of pituitaries; again, the other set served as a control. ACTH released by the pituitary halves, as well as any ACTH-like activity in the SME extract, was measured in a continuous flow, automated, *in vitro* superfusion apparatus containing adrenal quarters. This assay is described in detail in ref. 3.

Stalk ME extract of Brattleboro rats ($n = 7$) contains 98% ($\pm$ s.e., 12) of the CRF-like activity of normal Sprague-Dawley rats ($n = 6$) and 96% ($\pm$ s.e., 12) of that of normal Long-Evans rats ($n = 4$). Therefore, we conclude that Brattleboro rats, which lack AVP and its associated neurophysin⁵⁻⁷, contain essentially normal amounts of hypothalamic CRF activity, and, presumably, normal amounts of CRF. The only other published observation which shows a dramatic reduction ($\sim 60\%$) of CRF-like activity in SME extract from Brattleboro rats uses a dispersed cell pituitary assay system⁸, as do Gillies and Lowry. All workers seem to agree, however, that the Brattleboro rat can respond to stress with the secretion of near-normal levels of corticosterone⁸⁻¹⁰. Watkins observed a positive immunoperoxidase staining reaction in the paraventricular and supraoptic nuclei of Brattleboro rats when anti-[8-lysine]-vasopressin serum was used¹¹. Lutz-Bucher *et al.* have concluded that the anterior pituitary contains receptor sites for vasopressin which are distinct from

those for hypothalamic CRF¹². In this context, it is interesting that Zimmerman has reported immunological evidence for another peptide in Brattleboro rats which cross-reacts weakly with anti-vasopressin¹³ and may be related to the physiological CRF.

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GILLIES AND LOWRY REPLY—The above response to our paper¹ describes an experiment in which no difference was observed between the CRF activity of Brattleboro hypothalamic extracts and that of normal rats. As Brattleboro rats are deficient in vasopressin (VP), the authors conclude that VP is not CRF. However, our recent work with extracts of stalk-median eminence (SME) from Brattleboro rats² has added support to our idea that CRF is modulated VP. We found that Brattleboro SME contained approximately one fifth the CRF activity found in normal SME. Similar observations have been made using both isolated pituitary cells³ and pituitary quarters⁴.

Gel filtration of Brattleboro SME revealed only two small discrete peaks of CRF bioactivity corresponding in position and size to the Vo and Ns peaks found after chromatography of normal rat SME¹. The third and major CRF peak found in normal rat SME, which coelutes with synthetic AVP and resembles AVP in our immunoassay and CRF bioassay, was absent from the Brattleboro chromatogram. Furthermore, addition of synthetic AVP in amounts equivalent to that found

in normal SME (10 ng AVP per SME⁵) to extracts of Brattleboro SME completely restored the CRF activity to that of normals². This observation has now been confirmed in a different assay system⁴. These results coupled with the facts that (1) we have consistently been able to combine the Vo and Ns CRF peaks from the chromatogram of normal SME with either the major AVP-CRF peak or equivalent amounts of synthetic AVP to restore full CRF bioactivity¹ and (2) no analogue of VP has been found with CRF bioactivity greater than AVP^{5,6}, suggest to us that we have ample support for our conclusion that the AVP-CRF peak is true vasopressin, and not merely related in structure to it.

The above response cites Zimmerman's preliminary observation on the possibility of a vasopressin-like peptide in the Brattleboro rat which may be related to CRF. In a subsequent report⁷, however, it was stated that homozygous Brattleboro rats lack VP and its associated neurophysin and the occasional staining of some cells in the SON and PVN of these rats with their antiserum to AVP "appears to be due to slight reactivity of this antiserum to oxytocin". It should also be noted that the antiserum used by Watkins⁸ had 14% cross-reactivity with oxytocin and (8-arginine)-VP, so one cannot exclude the possibility of a positive immunohistochemical reaction in the SON and PVN of homozygous Brattleboro rats being due to oxytocin.

The immunocytochemical studies of Stillman *et al.*⁹, showed that VP and its associated neurophysin are stimulated by adrenalectomy, and their content in pathways to the portal capillaries is regulated by glucocorticoids, suggesting an important role for VP in corticotropin release.

As Pearlmutter *et al.* state above, all agree that Brattleboro rats are able to respond to stress. However, results of *in vivo* experiments are contradictory as to whether this stress response, as measured by adrenal activity, is normal¹⁰ or significantly subnormal¹¹⁻¹³ and, if an abnormality exists, whether it is at the hypothalamic, pituitary or adrenal level. We feel that many of the contradictions from *in vivo* experiments could be clarified, and a truer measure of CRF release in response to stress would be made, if ACTH release were the parameter measured, because supramaximal stress stimuli may lead to the release of CRF (the non-VP components in Brattleboro rats) and consequently ACTH, in amounts exceeding those necessary for maximal stimulation of the adrenal glands. This could explain why Yates *et al.*¹² observed that a deficiency in the stress response of homozygous Brattleboro rats was evident only when submaximal stresses were investigated.

We also suggest that the bioassay system used in *in vitro* experiments

contributes to this controversy on the role of VP as a CRF. First, isolated cell systems in general, and especially the perfused isolated cell system, as bioassays for CRF are far more sensitive than those using pituitary halves or quarters [we were able to demonstrate a threefold potentiation of 0.025 Brattleboro SME per ml by 0.25 ng AVP per ml (ref. 2)]. This must account for the fact that we were able to observe discrete peaks of CRF bioactivity in the chromatogram of extracts of small numbers of rat SME, and identify one peak as VP¹. Second, fresh excised rat anterior pituitaries contain significant amounts of VP¹⁵ which disappear from the tissue only after incubation for 3 h, suggesting specific binding to receptors. Therefore, in bioassays which use freshly removed tissue or incubated tissue which has been previously stimulated with solutions containing VP, such as a hypothalamic extract (as in the bioassay system used by Pearlmutter *et al.*⁶), it is possible that no difference would be found between the CRF activity of Brattleboro and normal rat SME, as the VP component of CRF would already be in the tissue. It is interesting to note here that Buckingham and Leach⁴, who use a CRF bioassay involving rat anterior pituitary quarters coupled with the cytochemical bioassay for ACTH¹⁶, have reported diminished CRF bioactivity in Brattleboro SME. As well as the difference in the ACTH bioassays used, the discrepancy with the findings of Pearlmutter *et al.* may be due to the duration of static incubation of extracts with anterior pituitary tissue—15 min for Buckingham and Hodges¹⁶ and 60 min for Pearlmutter *et al.*⁶, compared with 2-3 min contact of stimuli with isolated cells in our perfused system. The net CRF bioactivity observed could depend on the breakdown of both CRF and ACTH in the incubation medium.

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BOOK REVIEWS

What the tortoise never said to Achilles*

Oliver Penrose

ACHILLES had overtaken the Tortoise, and had seated himself comfortably on its back. "So you've got to the end of our race — er, our book" said the Tortoise. "Even though it DOES consist of an infinite variety of self-referring statements, curious puns, paradoxes, and speculations? I thought some wisecrack or other had proved that the thing couldn't be done?"

"It can be done", said Achilles. "It HAS been done! *Solvitur legendo*. You see each pun or paradox only took HALF as long to read as the one before; and so . . ."

"But the Index", interrupted the Tortoise, "how did you finish THAT? Every time you came to the entry 'Achilles: mapped on to ant colony 318, 324' you surely had to turn back to the middle of the book, so how could you EVER finish?"

"That splendid index", murmured Achilles. "It's true I did have some trouble getting past the entry 'Zen Buddhism; computers and, 625 — 26' especially as one of the chapters is based on Zen kōans, but even so I'm sure I'd have beaten you if I hadn't spent so long trying to do that fascinating MIU puzzle in chapter 1".

"Oh yes", recalled the Tortoise, "given the string of letters MI, and the rules for producing new strings:

1. If your string ends with I, you can add a U at the end.
2. If your string is Mx, where x is any string, you can change it to Mxx.
3. If your string contains the sequence III, you can replace that sequence by U.
4. If your string contains the sequence UU, you can delete that sequence.

can you produce the string MU?"

"Well, I'm only a simple warrior", said Achilles "and I never did manage to produce MU, but I thought the puzzle was a BRILLIANT way of introducing the idea of a formal logical system."

"And formal logical systems are very important", said the Tortoise, "because the centrepiece of the book is a sketch of the proof of Gödel's theorem: that practically any formal logical system contains statements which can't be either proved or disproved within the system."

"Ah yes, that proof of Gödel's theorem", sighed Achilles. "Very entertaining, like the rest of the book, but even after reading it an INFINITE number of times I still don't think I understand it."

Gödel, Escher and Bach: An Eternal Golden Braid — A Metaphorical Fugue on minds and machines in the spirit of Lewis Carroll. By D. R. Hofstadter. Pp.777. (Basic Books: New York, 1978; Harvester: Brighton, UK, 1979.) \$18.50; £10.50.



"Each reading taking only half as long as the previous one, of course", mused the Tortoise. "But I think the author's intention was not so much to give a crystal-clear explanation of Gödel's proof (he might possibly have done that better by making more use of Cantor's diagonal construction) as to exploit the idea of self-reference to the full. So he gives, as an analogue of Gödel's undecidable formal proposition, the following English statement: 'yields falsehood when preceded by its quotation' yields falsehood when preceded by its quotation."

"That must be the grandmother of all confusing self-referring sentences", said Achilles, mopping his brow. "I never DID manage to decide whether that one is true or not. But rather than get worried about it I turned to the Escher lithographs which he uses so delightfully to illustrate many of his ideas — for example, the picture of someone in an art gallery looking at a picture of a town which contains an art gallery which contains . . ."

"Do you mind", interrupted the Tortoise, "you are sticking your sword into my foot."

"MIND?", said Achilles, waving the sword excitedly. "That's just the kind of thing the rest of the book is about. What is a mind? Where is meaning located? How is knowledge represented? Can a computer

be conscious? Can animals with very primitive nervous systems be said to think? . . ."

"More easily than featherbrained warriors", muttered the Tortoise testily.

"Do you remember the description in the book of Terry Winograd's artificial intelligence device SHRDLU," went on Achilles, as if the Tortoise had not spoken, "which can move solid objects around in response to commands like 'find a block which is taller than the one you are holding and put it into the box'?"

"With comments by 'the newly developed human being, Dr Tony Earrwig' who seems to be related in a mixed-up way to SHRDLU's creator" added the Tortoise. "By the way, if you had to read the chapter about Gödel's theorem so often, I'm amazed that you finished the book so quickly."

"Well, I have to admit", said Achilles, "that to help me catch up with you, I only read every other chapter. The book still seemed to make sense though."

"What?" The Tortoise was horrified. "You missed the best part. Those chapters are all about US. And you missed my wonderful invention, the Tortoise property, enjoyed by every number that is the difference of two primes".

"Are you sure you don't mean the Goldbach property, which is that the number is the SUM of two primes?"

"No, the difference. The Goldbach property is VERY dull: you can always tell in a finite number of steps whether a given number has it or not, whereas if a number does not have the Tortoise property you could go on trying primes for ever. Besides that", continued the Tortoise, "you missed some extremely ingenious dialogues, which are verbal imitations of contrapuntal devices used by Bach in his much-loved works such as the Musical Offering. They provide welcome points of relaxation between the more serious chapters that you did read."

"With Bach as a model, the book must be even better constructed than I thought", said Achilles. "And the author's enthusiasm certainly carries you along as you read. But don't I see our mixed-up relatives Les Cahil and Sir Otto E. over there? Let's go and join them". □

*With apologies to Lewis Carroll "What the Tortoise said to Achilles", *Mind*, n.s.4 (1895), pp.278-80, reprinted in pp.43-45 of the book reviewed here.

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Interferometric spectroscopy

E. R. Pike

The Principles of Interferometric Spectroscopy. By John Chamberlain. Pp. 347. (Wiley: Chichester, UK, 1979) £20.

THIS book is the result of the completion, collection and revision (to include up-to-date advances, by Chantry and Stone) of material left at the time of John Chamberlain's death in 1974. It represents an attempt to bring together the whole field of interferometric spectroscopy at a practical working level in a single self-contained and readily accessible fashion.

As a knowledgeable and able practitioner Chamberlain leads authoritatively from the work of Michelson through a historical introductory chapter to an exposition of introductory theory in chapters 2, 3 and 4. These three chapters cover the ground of the Fourier transform, sampling theory and related topics, the mathematical description of the light field, including the concepts of the analytic signal, coherence and the coherency matrix, and the theory of the practical interference function. The fast Fourier transform is treated in the book's final chapter. Much of this didactic material is, of course, readily available on the bookshelf but as presented here forms a particularly thorough and logical operational approach. A few purist quibbles can be raised here and there as, for example, on page 73 where it is stated that "Physically, the electric and magnetic wave-fields are real quantities . . .". Are

they? In this book the only signals implicitly used are photon annihilation rates at the detector. If we were to measure an electron detection rate, say, in another branch of physics, would we assert that they derived from a real Schrödinger wave?

The theory of transform spectroscopy forms the core of the text in chapters 5, 6 and 7. Here are treated, respectively, the measurement of spectral line-shapes from the visibility curves, then non-dispersive and finally dispersive spectroscopy. Again, as in the previous section, the work is notable for its thoroughness and logical development. Full details are given of the effects and practical methods of dealing with them of truncation, sampling and phase errors. Apodisation schemes, for example, are reviewed in considerable detail.

A general information-theoretic approach is absent. The Fourier transform is taken for granted to be the magic requirement to derive what went in from what came out. The method of interferometric spectroscopy, however, is an ill-conditioned first-order Fredholm problem and thus insoluble without a 'model' for the inversion to inject sufficient extra *a priori* information to remove the ill-conditioning. The Fourier transform, viewed in this light, performs a least-squares fit to a model spectrum consisting of a truncated sum of cosine waves. Although this provides an extremely practical approach to retrieving such information about the spectrum as is transmitted by the instrument it surely does not squeeze out the last drop, and a discussion on these more general lines would have been interesting.

A competent chapter 8 is devoted to various experimental considerations such as finite apertures, imperfect mirrors,

modulation schemes and rapid scanning. Perhaps in experimental matters the rival book of R.J. Bell (*Introductory Fourier Transform Spectroscopy*, Academic Press, 1972) is more extensive.

The importance of interferometric spectroscopy derives in large part from the so-called Fellgett advantage, and it was a disappointment to the reviewer to find, yet again, both in the introductory chapter and in chapter 9 on noise, the commonly propagated fallacious explanation that the improvement is due to seeing all components of the spectrum at once. To a mathematician the nature of a function does not change in its Sturm-Liouville transform and one would naturally ask why the grating is not superior because it sees all delay times at once! This (in his own words) "difficulty in navigating the trickier fine points" shipwrecks him on the erroneous conclusion (page 263) that a multiplex advantage does not exist in the photon noise-limited region, although, apparently without its relevance fully sinking in, the result of Kahn (correct within a factor of 2) is given on page 302. He founders also on page 299, getting completely wrong the intensity and photoelectron fluctuations for quasi-monochromatic light. "Gaussian" and "Bose-Einstein" should be replaced by "negative-exponential" and "geometric" in this discussion.

Apart from these blemishes, the first of which seems to be indigenous to the subject, the book can be recommended to anyone entering this field, even at the specialist price of £20. □

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Small mammal ecology

Ian Linn

Ecology of Small Mammals. Edited by D.M. Stoddart. Pp. 386. (Chapman and Hall: London, 1979.) £15.

THIS book is an interesting and worthwhile attempt, in a series of essays by specialist authors, to review the state of the art in small mammal ecology and to indicate the areas in which this field of study still requires more intensive investigation. As a review it succeeds quite well. The individual essays reveal, in their expertise, the undoubted skill of the authors in their special fields, and the general coverage makes the whole exercise useful. Nevertheless, there is a lot of bias in this coverage, and it seems to me that the Editor might have exerted more discipline on his contributors, to ensure that the subject was

dealt with more evenly.

Thus, the emphasis of the book is firmly laid on the ecology of the rodents of north temperate regions. This emphasis is a simple reflection of the animals and regions about which most is known, but the Editor on the first page of his preface defines small mammals as those weighing less than 5 kg, and there are plenty of insectivores, carnivores and lagomorphs in that category, some smaller primates and even a few artiodactyls. There are honourable mentions of some of these here and there, and a couple of special chapters devoted to bats and marsupials, but more is known than would appear from the text. Even in the case of rodents, which are well covered on the whole, the special problems of tropical animals receive less attention than I would have expected.

If one continues to examine the book with comprehensive coverage in mind, some regrettable general lacunae are to be found. Here I would pick out particularly the topic of behavioural ecology. It is a pity that this aspect of the biology of mammals,

which throws such considerable light on their place in the ecosystem, is treated so sparsely. Similarly it is a matter for regret that food habits and feeding ecology are only briefly mentioned.

Perhaps it is too much to expect comprehensiveness in a book of this size, and it should simply be seen as a collection of more or less separate essays, reflecting the views of the authors about certain undoubtedly important areas of knowledge. As such the book is valuable, and can be read with profit by any serious ecologist, and by many others (doctors, veterinarians, pest controllers) whose interest in mammal ecology is more marginal. Professor George Varley once remarked to me that it was time the mammal ecologists got their subject to the same level as the entomologists. Perhaps we still have some way to go, but this book is proof that tremendous strides have been made towards this desirable goal. □

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Realm of pericyclic reactions

C. W. Bird

Organic Reactions and Orbital Symmetry. By T.L. Gilchrist and R.C. Storr. Second edition. Pp.311. (Cambridge University Press: Cambridge, 1979.) Hardback £25; paperback £8.50.

THE application of the concept of conservation of orbital symmetry in concerted reactions has had a profound effect upon the development of organic chemistry since its independent enunciation in 1965 by the late R. B. Woodward and R. Hoffmann, and H. C. Longuet-Higgins and E. W. Abrahamson. Not only did the concept facilitate the rationalisation of much knowledge, but more importantly it proved to have considerable predictive capability. The first edition of this book in 1972 provided a timely exposition of the rapidly expanding area, following hard upon the heels of the proselytising 'little blue book' of Woodward and Hoffmann with its peremptory claim "Violations. There are none!".

The reader of this most welcome second edition will search in vain for the dénouement usually visited upon such seeming arrogance by Murphy's Law. He will, however, find a lucid account of the

theoretical origins and multifarious applications of this concept. A résumé of the chapter headings provides a guide to the topics covered: classification and investigation of reaction mechanisms (20 pages); theory of concerted reactions (23); electrocyclic reactions (26); cycloadditions: introduction (36); cycloadditions and eliminations involving six electrons (57); other cycloadditions (69); and sigmatropic rearrangements and related reactions (63). While providing in a largely non-mathematical form accounts of the various alternative approaches to the underlying theory, stress is now placed upon the particular merits of the frontier molecular orbital approach. However, as might be expected of practising experimental chemists, the authors have emphasised throughout the synthetic applications of these pericyclic reactions. They have also succeeded admirably in their intention of providing a perceptive up-to-date introduction to the subject rather than an exhaustive survey, although extensive references to other books, review articles and original papers are provided for those requiring further information.

Like its predecessor this book provides an excellent introduction to the realm of pericyclic reactions, and can be unhesitatingly recommended to both neophyte and pedagogue. □

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Laser speckle

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Laser Speckle and Applications in Optics. By M. Françon. Pp.161. (Academic: New York, London and San Francisco, 1979.) \$16.

THIS book gives a concise, well ordered account of the origin and nature of speckle and of how it can be applied usefully. There are many illustrations in the book (158 in total), and with an absence of all but the most trivial mathematics, it makes pleasant and comfortable reading. The book has ten chapters, each broken into about six sections.

Chapter 1 begins by considering the image of a point source, and from this very humble starting point it progresses to deduce the image of many point sources, that is, a speckle pattern. Chapters 2 to 5 consider the effects of changing some of the variables which govern the speckle patterns. In addition to many more, consideration is given to such things as changing the plane of observation of the speckle pattern and changing the orientation and wavelength of the illumination. To one who might regard speckle patterns as being random and

ungoverned by sensible laws, the results outlined in these chapters will come as a pleasant surprise. There are many ways in which speckle patterns behave simply and predictably, and the author has brought this out very clearly. The material comprising these chapters forms a basis for the applications which are dealt with in the remaining chapters.

Image processing is considered in chapter 6 and, along with several others, techniques are described for extracting the difference between two images, as are techniques for coding and decoding images. Chapter 7 is concerned with the study of displacement and deformation using speckle patterns. Chapter 8 concerns speckle in astronomy and the elegant method of Labeyrie for gaining diffraction limited performance from the largest telescopes. Chapter 9 deals with the measurement of surface roughness and Chapter 10 deals with various other applications of speckle patterns which do not fall within the previous categories.

The book presents a simple concise account of the subject. Researchers will find it very useful as a book for first reference; and, for those who wish to go deeper into the subject, an extensive list of references is given. □

T. S. McKechnie is in the Department of Mechanical Engineering at Loughborough University, Loughborough, UK.

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Geophysics of Mars

Garry E. Hunt

Geophysics of Mars. Developments in Solar System and Space Science, Vol.4. By R. A. Wells. Pp.678. (Elsevier Scientific: Amsterdam, Oxford and New York, 1979.) \$87.75; Dfl. 180.

FOR centuries, the planet Mars has been the centre of intrigue for Mankind. Across the cold icy darkness of space, there has always been speculation that life could have developed on our planetary neighbour. In recent years, detailed observations from spacecraft have added to our measurements of the Martian environment by Earth-based telescopes, so that we are now building a fairly comprehensive picture of the planet now, and some interesting indications of its past. In this monograph, Dr Wells has produced a huge text which tries to put into perspective our current understanding of the geophysics of Mars. I believe he has largely succeeded in a well written and carefully illustrated text.

Naturally, the structure of the text and its content must have some personal biases, but fortunately all of the eight chapters have a wealth of references to supplement the material, so that the reader will not feel that his understanding of Mars is too strongly influenced by the author's own views. We are led into the discussions of Mars through the physical interaction of the atmosphere and surface. The importance of the Martian atmosphere in shaping some of the surface features and the minor constituents in providing

information on the past history of the planet are reviewed. The chapter continues by introducing the basic spectroscopic matters related to the detection of atmospheric constituents such as CO₂, CO, O₃ and H₂O, their variability, and the basic techniques of determining the surface pressure and aerosol content of the atmosphere. Mars does possess a very exciting meteorology and I personally think it is unfortunate that this characteristic has not been highlighted more. There is some discussion of the cloud systems seen on the planet, but those seen by Viking in the Tharsis area, such as bore wave clouds, are unfortunately incomplete. The discussion of the dust storm generation misses out the fundamental observations by the Viking imaging and infrared instruments which have clearly shown that separate mechanisms are responsible for the preperihelion and perihelion storms.

Two of the most controversial aspects of Mars are concerned with the obscuration of the dark surface markings at short wavelengths and the progressive, seasonal, cyclic increase in their darkness at long wavelengths. These aspects are more familiarly known as the blue haze and the seasonal wave of darkening, respectively. A complete chapter is devoted to these matters. The discussions are very thorough. The reader will also be introduced to some of the problems and applications of Mie scattering theory in a simple and effective way which is always useful.

The next three chapters deal with different aspects of the basic surface properties of the planet. The geological aspects of Mars are built up from our early telescopic observations and subsequently

improved with the spacecraft data through the 1960s until the present Viking data is available. A brief discussion is given of the problem of estimating the age of a surface by crater counting, but I am not sure whether the reader will necessarily know which set of results he should believe at this stage. The remaining two chapters of this sequence cover the Martian topography and the linear features. It may be sad for some readers that Lowell's infamous canals do not relate to any of the Martian features. However, this situation is in itself a valuable calibration, and a direct indication of the care we must take in interpreting visual observations.

Two further chapters cover the internal structure of the planet, mantle convection and the Martian moons, Phobos and Deimos. The monograph concludes with a detailed discussion of the first few months of the Viking view of Mars. As a result, a great deal of the seasonal aspects, and all the annual variations of the atmosphere, are not included.

This book has been written with great care, and there is every indication that the author has devoted himself to this task of placing on record the geophysics of Mars. I certainly believe that it is a valuable addition to the discussion of the Martian environment, and is well supplemented by a large number of high quality diagrams and photographs. But how many people, or indeed libraries, can really afford £41? I wish publishers would try to be a little more realistic in their prices; then good books, such as this one, would reach a much bigger audience. □

Garry E. Hunt is Head of the Laboratory for Planetary Atmospheres, Department of Physics and Astronomy, University College, London, UK.

Electron microscopical techniques

S.J. Kirchanski

Electron Microscopy and Cytochemistry of Plant Cells. By J.L. Hall. Pp.444. (Elsevier/North Holland: Amsterdam and New York, 1979.) \$46.25; Dfl.95.

THIS book is indeed a pleasant surprise; all too often edited volumes are very uneven in quality and contain unfocused or inappropriate chapters. On the contrary, in this work all the chapters are of good quality and contribute an overall unified aspect to the volume. There are seven chapters which cover preparation of thin sections, enzyme cytochemistry, labelled antibodies and lectins, ion localization, autoradiography, freeze fracture and scanning electron microscopy.

Each chapter begins with a useful outline of its contents. The reader is introduced to the techniques covered in the particular chapter, and in some cases a brief historical review of the subject is undertaken. This is followed by detailed descriptions of the various technical steps along with informative discussions of alternate methods in most cases. Each chapter is thoroughly illustrated and has a good, current bibliography. The overall quality of printing is high and the micrographs are well reproduced. The chapters on thin sections, cytochemistry and lectins each end with appendices of methods and recipes which will doubtless prove extremely popular with most researchers.

Of course, given the space restraints of the volume, it is not possible for each chapter to be absolutely complete. Different workers will undoubtedly notice different omissions. In the chapter on thin sectioning I was pleased by the detailed treatment of staining (although I believe that Reynolds should have been credited with the lead citrate procedure that was

presented), but I felt that insufficient discussion was given to the value of low viscosity resins for embedding plant materials. In the same way, though I felt that the historical treatment of freeze fracture and the descriptions of standard freeze-fracture methods were masterful, I think that insufficient coverage was given to low-temperature, rapid freezing (Heuser-liquid helium methods) and the revolution that rapid freezing is currently causing in freeze fracture. Nevertheless, this is an excellent volume and should be available in all biological research libraries. Although the material is aimed towards plant biologists, it is of use and value to all biological electron microscopists. In spite of the volume's rather high price, I predict that many researchers will purchase it as a personal reference book. □

S.J. Kirchanski is a Postdoctoral Research Fellow and Lecturer at Harvard University Biological Laboratories, Cambridge, Massachusetts.

OBITUARY

L. P. Garrod

LAWRENCE PAUL GARROD, emeritus professor of bacteriology in the University of London died on 11 September 1979, aged 84. He was born on 7 September 1895. After school at Sidcot, Garrod's medical training at King's College, Cambridge and St. Bartholomew's Hospital was interrupted by service in 1917 and 1918 as a Surgeon Sub-Lieutenant, RNVR. At Bart's, his career as a student was outstanding and he won the Senior Scholarship in Medicine. In due course, after qualification, he became Chief Assistant to Dr Morley Fletcher and at that stage he seemed bound for the staff of the hospital as a consultant physician. But in 1925 he turned to pathology and then bacteriology, being appointed Professor of Bacteriology in the University of London in 1936, a post which he held with that of Bacteriologist to the hospital, until his retirement in 1961.

At Bart's he was a familiar and much respected figure. To the thousands of Bart's doctors who were taught by him during his time, he will probably best be remembered for his lectures and the associated practical classes at which he was a meticulous attender. A very clear logical speaker, his lectures were illustrated by a series of anecdotes and comments that held the attention of his students. To the hospital staff, his wide clinical and laboratory experience was always available. His opinion was always worth having and was much sought.

To a much larger audience, Garrod was known for his research and for his writing. Before the last war he became interested in disinfection and sterilisation, a subject that attracts the interest of few doctors, and he rapidly became one of the leading authorities in the country, with his name attached to one of the standard tests for disinfectants. This early experience stood him in good stead with the discovery of sulphonamides and then antibiotics, and a flow of papers began from his laboratory, many with his assistant, Miss Pamela Waterworth, dealing with chemotherapy in general and the properties and uses of each new antibiotic as it emerged. He played an important part in the Medical Research Council's trial of the penicillin treatment of subacute bacterial endocarditis, that was to set the pattern for many subsequent clinical trials.

As a writer, Garrod's contributions, both recognized and anonymous, were immense. He edited the *British Journal of Experimental Pathology* for many years and his close association with the *British Medical Journal* ran from 1931 until he died; he served on various committees and was at one time Chairman of the BMJ Journal Committee. When he retired from Bart's, the then Editor of the *British*

Medical Journal, writing in the Hospital Journal, said that "it is difficult for me to say just how much we on the staff of the BMJ — and its readers — owe to Garrod. He has written book reviews, leading articles, annotations, answers to questions, all with effortless ease, precision of style, accuracy of fact, and a certain marked honesty that has been vastly refreshing."

Apart from his other writing, he was co-author of three books. First, before the war, *Recent Advances in Pathology* with Geoffrey Hadfield and then after the war, *Antibiotic and Chemotherapy*, first with Mary Barber and later with Francis O'Grady, and *Hospital Infection* with R.E.O. Williams and others. Retirement did not slow his pen, and he continued his work for the BMJ as well as answering the many invitations to speak that he received from this country, Europe and the United States.

Professional recognition came his way by honours and appointments. His views were sought as a committee member by the Department of Health, the Medical Research Council and World Health Organization. He examined for the Universities of London, Oxford and Cambridge. He was president of his section of the Royal Society of Medicine, Vice-President of the British Medical Association, President of the Institute of Medical Laboratory Technology, Consultant in Antibiotics to the Army, and on retirement, Honorary Consultant in Chemotherapy to the Royal Postgraduate Medical School. The University of Louvain conferred the title of Honorary Alumnus on him, Glasgow awarded him an Honorary Doctorate of Law and the Royal College of Pathologists elected him an Honorary Fellow.

R.A. Shooter

John Raymont

THE sudden death of Professor J.E.G. Raymont on 30 August 1979, at the age of 64, has deprived the world's community of marine scientists of a respected member and the field of zooplankton of one of its most distinguished students.

His interest in zooplankton began at University College, Exeter where he took first class honours in zoology, and was soon reinforced during 1937-38 when he held a Henry Fellowship at Harvard and spent the summer of 1937 at Woods Hole. After a brief return to Exeter as assistant lecturer he was appointed Lecturer in Zoology in Edinburgh in 1939. There, in conjunction with Sheina Marshall, F. Gross and A.P. Orr, a study was commenced on the effects on plankton and fish productivity of fertilising sea lochs, which was a precursor to marine fish farming in enclosed waters. During this period he met and married Brigit Sloan,

who remained his constant companion at home and colleague at work in an enviably happy marriage.

In 1946 John Raymont moved back to the south to take up the Chair of Zoology at Southampton at the early age of 31. A generous grant from the Goldsmiths' Company enabled the purchase of the department's first boat, *Aurelia*, and his attention, after a brief period of working with other organisms, turned back to plankton. This was an era of public and scientific interest in the possibility of utilising plankton directly or indirectly as food organisms and he initiated long-term studies on the composition and physiology of zooplankton species. One of the researchers attracted by his studies in this field was a young Indian biologist, S. Krishnaswamy, who subsequently collaborated with John Raymont on several occasions. Partly through this connection and partly from international prestige gained as a result of his definitive work *Plankton and Productivity in the Oceans* (1963), John Raymont was invited under the auspices of the Indian U.G.C. and the British Council to advise on the establishment of the Centre of Advanced Marine Studies at Porto Novo. Thus began a long association with the training of oceanographers in many lands which culminated in membership of such bodies as the I.O.C. (Training, Education and Mutual Assistance), S.C.O.R., Nuffield Tropical Marine Biology Fellowship Panel and Chairmanship of the UNESCO Advisory Panel on International Marine Biology Centres.

At Southampton his academic career was crowned by appointments as Dean of the Faculty of Science 1961-1963 and Deputy Vice Chancellor 1966-68. His real ambitions lay, however, in a somewhat different direction and these were fulfilled in 1964 with the foundation of a department devoted to the study of marine science in all its aspects. Under his skilful guidance the staff of this multidisciplinary department were welded into a coherent and integrated unit in spite of their disparate research interests. The department quickly gained the international reputation which will live on as a monument to John Raymont's skills as a quiet unifier.

His international commitments, research culminating in some 60 papers, and administration came nowhere near exhausting his energies and over the years he was *inter alia* an adviser to the C.E.G.B. on matters relating to warmed water release, a member of the British National Committee for Oceanic Research and the Royal Society Pollution Study Group, member of the Councils of the Fresh Water Biological Association, Marine Biological Association and National Institute of Oceanography. As Chairman of the

Planning and Development Committee of the Wessex Regional Hospital Board and later as Vice Chairman of the Hampshire Area Health Authority, he was deeply involved in the expansion of hospital services in the area.

He was honoured by the award of the OBE in 1978 and earlier by election to a Fellowship of the Indian Academy of Sciences.

Marine scientists the world over will grieve the loss of a man who by quiet example was able to foster participation between research workers at every level, of different interests and from many nations.

A.P.M. Lockwood

Jack Tizard

PROFESSOR JACK TIZARD died in London aged sixty, on 2 August 1979, as a result of cancer found to be inoperable eight months earlier. So passed one of the foremost post-war research workers in mental retardation and child development.

Born a New Zealander, Jack graduated there and after overseas Army services undertook a postgraduate degree at Oxford where he met his wife. In 1948 he joined the Medical Research Council's Social Psychiatry Research Unit at the Institute of Psychiatry in London, and with his colleague Neil O'Connor was directed by Professor Aubrey Lewis into the neglected field of mental deficiency. He was appalled by the situation he found in hospitals for the mentally defective which in those days were used as places where socially disadvantaged young people could be put away. As a fearless man he said so, among other things challenging the medical model which was enshrined in the 1913 Mental Deficiency Act. Tizard and O'Connor set about showing that industrial habilitation leading to fairly rapid discharge from care was a viable possibility. Their pioneering studies on the mildly retarded were brought together in 1956 in an important book *The Social Problem of Mental Deficiency*.

However, there was more to do. There remained the moderately and severely retarded who were on the whole considered unemployable in even the simplest capacity. In a series of innovative experiments it was shown that young adults with intelligence quotients in the thirties and forties could in certain circumstances learn, respond to incentives, retain their learning and transfer it to rather different situations. Others built upon and extended this work which cast new light on our potential for helping the more severely disabled, and their own potential for change.

Jack himself was ready to move on, and with the help of Dr Leslie Hilliard transferred a number of mentally handicapped children out of the Fountain Hospital and into a country house which resembled a boarding school. In 1961 he

published *The Mentally Handicapped and Their Families* (with J.C. Grad) and in 1964 *Community Services for the Mentally Handicapped*. His belief that the quality of the social structure and organization of any community is a major determinant of development and behaviour of its members led him into the field of social policy.

In 1964 Jack was appointed to the first Chair in Child Development at the University of London; his outstanding contribution to the study of mental sub-normality was acknowledged in 1968 when, jointly with Neil O'Connor, he received the Kennedy International Scientific Award, to be followed in 1973 by the annual research award of the American Association on Mental Deficiency. In the early nineteen-seventies, as Research Professor, he founded The Thomas Coram Research Unit, a unique multi-disciplinary establishment in which medical and a variety of social scientists collaborated in policy-oriented research projects. His erudition, high intelligence and unusually attractive personality ensured that he became much in demand for committee work. An excellent and incisive chairman, he was invited to act in several different capacities for a large number of organizations including the Social Science Research Council, the Department of Health and Social Security, the Association for Child Psychology and Psychiatry, the WHO Expert Committee on Mental Health, and for the British Psychological Society as president. He was appointed CBE in 1973.

Despite his generous contribution to committees, his research continued to flourish, and a steady flow of books and articles appeared over the years on a wide variety of topics relating to children. Jack Tizard was an example of that unusually effective individual, a radical thinker accepted by the Establishment.

In the final eight months, much of it in pain, Jack's bearing befitted his life. Fortitude, equanimity and hard work were his daily purposes; his courage was well matched by that of Barbara, his wife. Their long, happy marriage must have given both a background of security, and contributed in no small measure to the success each enjoyed.

To say of a man that "we shall not see him like again" is often a sentimental exaggeration; in the case of Jack Tizard, this is not the case. Those hundreds of us who knew him personally must feel a deep sense of loss at his passing.

Ann Clarke and Alan Clarke

Marcel Florkin

BORN in Liège, Belgium in the first year of the new century (15 August 1900), Marcel Florkin died in Liège on 3 May 1979. His early education and his medical degree were also at Liège, but thereafter he gained international recognition through his con-

tributions in the areas of invertebrate physiology, comparative biochemistry and physiology, biochemical evolution, and origin of life, as well as through numerous roles in the development of international aspects of biochemistry.

Florkin's interest in physiology and biochemistry were increased by study in the laboratories of such eminent scientists as Joseph Barcroft, Richard Kuhn, Heinrich Wieland, and Edwin Cohn, following which he spent 36 years in teaching and research at the University of Liège, interrupted by visiting professorships at Duke University and the Universities of Washington and Oregon.

Perhaps the most compelling urge of his life in science was to extend his interests and contributions beyond ordinary limits, into broadly related areas of history, comparative aspects of physiology and biochemistry, evolution, and the nature of men who have contributed uniquely to the field of biological science. In addition to his numerous individual contributions, he undertook prodigious tasks of assembling as senior editor the world's contributions in multi-volume collections in various fields. Witness the ten volumes of *Chemical Zoology*, the seven volumes of *Comparative Biochemistry* (Florkin and Mason), and the 34 volumes of *Comprehensive Biochemistry* (Florkin and Stotz). The last five volumes of the latter series were devoted to the *History of Biochemistry*, his own contribution, and constituting written testimony to his critical and intimate knowledge of the great events in biochemistry. Included in his *History* is a remarkable collection of photographs of pioneers in biochemistry, itself a notable tribute to his perseverance and human touch.

Florkin contributed greatly to the role of biochemistry at the international level. In 1952 he became chairman of the International Committee of Biochemistry, and in 1955 was named the first President of the newly formed International Union of Biochemistry, following which he was instrumental in gaining admission of the newly formed Union into the International Council of Scientific Unions. As first Treasurer of the Union, the writer can attest to the diplomatic skills and warm spirit of negotiation displayed by Professor Florkin.

Marcel Florkin gained many honors and awards in his active life, including honorary membership in the Royal Institution of Great Britain and honorary fellowship in the Royal Society of Edinburgh. Yet in his busy life he maintained an active interest in the arts, and seldom missed an opportunity in his travels to find a moment for a visit to the local art museums to view their recent acquisitions. It is not surprising that he was devoted to family and close friends, who, along with many scientific colleagues, will miss his great warmth and human spirit.

Elmer H. Stotz